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COMPOSITION AND APPEARANCE OF SOYBEAN PLANTS GROWN IN CULTURE SOLUTIONS EACH LACKING A DIFFERENT ESSENTIAL ELEMENT[1]

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Although considerable knowledge has accumulated by previous investigations regarding the functions of the essential mineral elements in plant growth, conclusive evidence concerning the rôle that any element plays at different stages of growth, as well as the extent to which it can be replaced by other elements, is still lacking. Even less information is at present available concerning the chemical composition of plants grown in culture solutions lacking one of the essential elements.

The following investigation was undertaken with two objects in view: First, to obtain information concerning changes that may take place in the absorption of nutrient elements by the soybean plant grown in culture solutions, due to the lack of one of the essential elements. Secondly, to study the physiological changes in the plant resulting from lack of the essential elements omitted singly from the otherwise complete culture solutions. Soybeans were selected because they are well adapted to growth in culture solutions and are not particularly susceptible to diseases. The Guelph variety was chosen on account of its short growth cycle.

HISTORICAL

While no attempt is here made to give a complete review of the literature on the subject, it is felt that a brief discussion of some outstanding earlier investigations may serve as a connecting link between the present experiments and those of other investigators. An extended review of the history and methods of culture solution work is given by Livingston (10), Livingston and Tottingham (11), Tottingham (19) and Shive (18), and a discussion of the functions of the essential elements is presented by Loew (12) and Pfeffer (15).

That the composition of the same species of plant is more or less variable, according to conditions, has been well established by the early investigators. Saussure (17) has shown that the composition of plant ash is not constant but varies with the nature of the soil and the age of the plant, while Wolff (20) has shown that selective absorption by plants could be altered by changing the proportion of salts in culture solutions.

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Nobbe and Siegert (16) made a comparative study of the composition of buckwheat plants grown in garden soil and in culture solutions. "The water plants had an ash content double that of the land plants." However, the seeds of the plants grown under the different experimental conditions were fairly constant in composition. Nobbe, Schröder, and Erdman as quoted by Johnson (6) used water cultures to make an elaborate study of the influence of potassium on the vegetative processes and found that in the absence of potassium, buckwheat "vegetated" for three months without any increase in weight.

Heiden (5) observed that maize and peas in culture solutions without lime lived only four weeks. In the absence of magnesium the same plant lived ten to twelve weeks and peas lived eight weeks. In solutions without phosphorus, such plants lived from eight to ten weeks. The plant shows the effects of the absence of lime, therefore, earlier than it does the lack of the other elements.

Burd (1) studied the composition of barley grown in two different soils. He observed striking similarities in the rate of absorption of nutrient elements by the plants. He calls attention to the remarkable losses or lack of potassium and nitrogen from the plant at an early stage of development, that is, at the beginning of the head formation, which are succeeded by renewed absorption at a later period.

Hartwell, Wheeler and Pember (3) and Hartwell and Pember (4) conclude that sodium caused no increase in growth of wheat seedling when an optimum amount of potassium was present in the culture solution, but if the deficiency of potassium was great enough to cause about 30 per cent depression in the green weight produced, the addition of sodium gave an increase in growth of 10 per cent or more within a period of two or three weeks. An extra addition of calcium did not, on the whole, increase the growth, either when used with an optimum or a deficient amount of potassium. They also show that a larger amount of potassium was left in the solution by the growing seedling when the potassium in the culture medium was supplemented by sodium. In other words, sodium in this case conserved potassium.

More recent experiments conducted by Lipman and Blair (7, 8, 9) show that the nitrogen content of the soybean plant is increased by the addition of lime. Neidig and Snyder (13) state that the increased amount of available nitrogen affects directly the protein content of wheat.

Dickson (2) found that a deficiency of potassium reduced the phosphorus content of the oat plant, while a deficiency of calcium increased it.

Loew (12) from his own investigations and from those of others, reaches the following conclusions: "Although magnesium salts cannot physiologically replace calcium, the former can fulfill their nourishing functions only in the presence of the latter. In the absence of calcium, magnesium even exerts an injurious action. In the absence of calcium, few root hairs develop, while a deficiency of magnesium does not interfere with root hair formation."

METHODS OF PROCEDURE

Two series of experiments were carried out in the greenhouse, the second an exact duplicate of the first but conducted at a different season of the year. In the first series, soybeans were grown in culture solutions from October 13, 1922, to January 6, 1923. The second series was conducted from January 17 to April 7, 1923, thus covering a period of some six months. Each series comprised 18 cultures, the plants being grown in 9 different culture solutions duplicated. In making up these solutions, Shive's solution R_5C_2 was used as a basis. The composition of these solutions is given in table 1.

Solution 1 of the table (Shive's R_5C_2) was used as a check in each case. Solutions 2 to 8 inclusive each lacked one of the essential ions, but no two solu-

tions lacked the same ion. The dropping of a single essential ion from the complete culture solution was accomplished in every case by replacing one salt in the check solution by another, one ion of which was different from that in the replaced salt but common to an ion already supplied by one or the other of the two remaining salts in the check solution. For example: solution 2 in table 1 was prepared by replacing $Ca(NO_3)_2$ with KNO_3, thus omitting Ca but supplying no new ion to the solution. The table indicates these substitutions and gives the H-ion concentrations of the resulting solutions, the concentrations being determined electrometrically. In solution 9 sodium was substituted for potassium by replacing KH_2PO_4 with NaH_2PO_4 in equivalent amounts. In the preparation of these solutions, all substitutions were made on the basis of equivalent grams per liter of the salts employed as the table indicates.

TABLE 1

Composition of the culture solutions salts per liter

CULTURE NUMBER	ELEMENT LACKING	KNO_3	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	K_2SO_4	$Mg(NO_3)_2$	$Ca(H_2PO_4)_2$	NaH_2PO_4	$FeSO_4$	pH VALUE
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.		
1*	....	....	0.8533	1.8060	2.4507	....	....	....	....	As required	4.57
2	Ca	0.8533	....	1.8060	2.4607	....	....	....	....	As required	4.70
3	Mg	....	0.8533	....	2.4507	1.8060	....	....	....	As required	4.65
4	K	....	0.8533	1.8060	....	....	....	2.4507	....	As required	3.31
5	N	....	....	1.8060	....	0.8533	....	2.4507	....	As required	4.16
6	P	2.4507	0.8533	1.8060	....	....	....	....	....	As required	5.27
7	S	....	0.8533	....	2.5507	....	1.8060	....	....	As required	4.77
8	Fe	....	0.8533	1.8060	2.4507	....	....	....	....	Omitted	4.57
9†	K	....	0.8533	1.8060	....	....	....	....	2.4507	As required	4.69

* Check.
† Na replacing K.

The seeds were germinated in sphagnum moss, and seedlings of uniform size were mounted in paraffined cork stoppers, as described by Tottingham (19), and transferred to the quart jars of colorless glass which were used as culture vessels. The jars were enclosed in thick manilla paper shells to exclude light. The solutions were renewed every $3\frac{1}{2}$ days throughout the growth period. The culture solutions were prepared as they were used from single 0.5 M stock solutions of all salts except $Ca(H_2PO_4)_2$, which on account of its low solubility was used as a stock solution in 0.1 M concentration. As the plants appeared to require it iron was added to each culture solution, except 9, in the form of a freshly prepared solution of $FeSO_4$ in concentration of 0.1 mgm. of iron per cubic centimeter.

Daily observations were made of each culture, and the physiological changes in roots and tops of the plants as well as in the general appearance were care-

fully studied.   When the plants had matured or when growth had ceased, the tops and roots of the plants from each culture were harvested separately, dried and weighed.   All the plant material from the same series was then mixed together, finely ground and stored in tightly covered bottles for chemical analysis.   Because of early injury sustained by the plants, some of the cultures produced a yield insufficient to permit complete analyses for all essential mineral constituents of the plants.   Analyses, therefore, for calcium, magnesium and nitrogen only were made, because in the absence of these elements the plants sustained the most pronounced injurious effects observed.   The methods recommended by the Association of official Agricultural Chemists (14) were used in all chemical analyses, all of which were made in duplicate.   The results presented in the tables are the averages of the duplicate determinations.

TABLE 2

Average yield of roots, tops and total dry matter from plants grown in check solution and in solutions lacking one essential element

CULTURE NUMBER	ELEMENT LACKING	ROOTS (12 PLANTS)			TOPS (12 PLANTS)			AVERAGE WEIGHT OF PLANTS
		Series 1	Series 2	Total	Series 1	Series 2	Total	
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
1*	...	1.50	2.01	3.51	13.64	10.45	24.09	2.30
2	Ca	0.09	0.21	0.31	0.92	1.03	1.95	0.19
3	Mg	0.38	0.41	0.79	2.71	2.24	4.95	0.48
4	K	0.44	0.37	0.81	1.79	1.33	3.12	0.33
5	N	0.18	0.25	0.43	0.73	1.13	1.86	0.20
6	P	0.91	1.25	2.16	6.73	7.46	14.19	1.40
7	S	0.64	1.03	1.67	4.05	6.68	10.73	1.30
8	Fe	1.14	0.85	1.99	9.41	5.43	14.84	1.40
9†	K	0.88	0.81	1.69	5.94	3.94	9.88	1.05

* Check.

† Na replacing K.

PHYSIOLOGICAL CHARACTERISTICS OF THE PLANTS

The plants were frequently observed and carefully studied during the entire growth period for the appearance of injury or any abnormalities resulting from the lack of an essential element in the culture solutions.   The occurrence of injury or of any changes from the normal growth phenomena was carefully noted, and particular care was taken to determine the exact time which elapsed after the plants were placed in the incomplete culture solutions until some injury or abnormal condition appeared.

Although the two series of cultures were conducted at different seasons of the year, the injuries and abnormal conditions that appeared were remarkably the same from corresponding cultures of the two series.   The numerical data relating to dry weight yields of tops and roots from each series are presented in table 2.   In the check culture the plants appeared perfectly normal throughout,

and produced pods with well-developed seeds. They were taller than those from any of the other solutions and of course produced the highest yield, giving an average of 2.30 gm. of dry matter per plant.

While the dry weight data of table 2 do not, of course, indicate the nature of the injury sustained by the plants, nor the peculiar characteristics resulting from the lack of any of the essential ions, they do indicate clearly the relative growth-retarding influence of the absence of the different essential ions singly from a culture medium such as was here used. Thus, judging from the average relative dry weight yields per plant, the degree of injury or growth retardation from the highest to the lowest, resulting from the absence of the essential ions singly from the medium, proceeds in the order of calcium, nitrogen, potassium, magnesium, sulfur, iron and phosphorus. This is clearly brought out by figure 1.

The plants grown in the solution which lacked calcium gave pronounced evidence of injury on the third day after the seedlings were transferred to the jars. The primary roots turned brown and growth of the laterals ceased. The primary root, however, continued to increase in length slowly for 7 days, when the plants wilted and died. The veins in the leaves turned brown and this was soon followed by curling of the entire leaf surface without further discoloration. The total yield from 12 plants was only 2.25 gm. of dry plant material, or an average of 0.19 gm. per plant, as compared with 2.30 gm. per plant from the check.

In the absence of magnesium, the tops of the plants were first affected. During the period of 8 days following the transfer of the plants to the solutions lacking magnesium, they showed no difference in appearance from those grown in the check solution. After this period, the upper surface of the leaves became covered with small brown spots and soon dropped. New leaves continued to be formed but these also soon became spotted and fell, so that the stems were always bare of leaves except at the tops. The roots appeared to grow normally during the first 20 days, after which a rapid loss in rigidity was observed. They became very soft and assumed a strikingly white appearance. The plants, however, continued to grow, producing a few small pods but no seeds. Growth stopped completely only after the plants had been 7 weeks in this solution. A total yield of 5.74 gm. of dry matter was obtained from the cultures of the two series, or an average of 0.48 gm. per plant as compared with a corresponding yield of 2.30 gm. from the check.

In solutions lacking potassium, the roots showed signs of injury before the tops were affected. The roots became thickened and showed distinct evidences of decay after having been 6 days in the solution. Very few lateral roots developed; these appeared near the base and were thick and white. The severe injury to the roots in this solution during the very early part of the growth period may have been due more to the high H-ion concentration (pH 3.31) than to the absence of-potassium, The tops, however, continued to grow slowly, the leaves maintaining a normal green color throughout a period of

7 weeks, after which the plants died. The leaves were small and the plants were dwarfed, as is indicated by the low yield of dry matter, which gave an average of only 0.33 gm. per plant, while the corresponding yield from the check was 2.30 gm. per plant.

When nitrogen was omitted from the culture solution, the effects upon the roots were similar to those observed in solutions where potassium was lacking, although the H-ion concentration of the nitrogen-free solution was considerably lower (pH 4.16) than of that lacking potassium. It appears, therefore, that the severe early injury to the roots in these two solutions may not be attributed wholly to the H-ion concentration of the solutions, but bears also a direct relation to the absence of the two vey important ions in question. This is emphasized by the fact that no such similarity was observed in the tops of the plants grown in these two solutions. The tops of the plants in the nitrogen-free solution were very slender and began to wilt on the eighth day in the solution. The plants were dead at the end of 2 weeks. No distinct chlorosis was observed at any time, the leaves becoming quite dry without discoloration. The average yield per plant was only 0.20 gm., as compared with a corresponding yield of 2.30 gm. from the check.

The plants grown in culture solutions from which phosphorus was omitted showed no injurious effects during the first 18 days, after which the roots assumed a brownish color. The leaves and stems grew to maturity showing the usual characteristics of normal plants, and the plants produced pods with a few seeds of good size and weight. It may be of interest to note, however, that none of the pods contained more than one seed and that the seeds were not viable. Another interesting fact observed was that these plants required less iron to keep the leaves green than the plants in any of the other solutions. A good green color was maintained in the leaves throughout the growth period by the addition of only 0.1 mgm. of iron per liter of solution, while from 0.4 mgm. to 0.6 mgm. per liter of solution was required for the check plants. This was probably due to the fact that in the absence of phosphorus, iron tends to precipitate less readily in these solutions. No wilting of the plants was observed at any time during the growth period, but from the time of blossoming to maturity, newly formed leaves showed small brown spots on the under surface. The plants were, of course, considerably smaller than those from the check, as is indicated by the dry-weight yield, which gave an average of 1.40 gm. per plant while the check gave a corresponding yield of 2.30 gm.

In solutions free from sulfur, the first abnormalities were observed in the leaves. After having grown normally for 2 weeks, the leaves gradually turned yellow and then became covered with brown spots. This was followed by browning of the roots and a rapid falling off of the growth rates of the plants in general. The green color of the leaves could not be brought back by increasing the amount of iron, which tends to show that the chlorosis in this case is not a question of iron deficiency, but of some other inhibiting factor in the chlorophyll formation. While an abundance of blossoms appeared, none

of the plants developed pods. The plants grew for 9 weeks, producing an average of 1.03 gm. of dry matter per plant as compared with 2.30 gm. per plant recorded for the check.

A marked difference was observed between plants grown in potassium-free solutions and where potassium was replaced by sodium. In the culture solution containing sodium but no potassium, the plants appeared normal until the time of blossoming. Soon after this the leaves showed distinct chlorosis and became covered with brown spots, a characteristic not observed in plants grown in the potassium-free solution. The leaves partially recovered from the chlorotic condition when the amount of iron in the solution was gradually increased, the leaves assuming a pale green color, indicating that the physiological disturbance was not the result of iron deficiency. At harvest time the roots were rigid and brown, resembling somewhat those grown in the phosphorus-free solution, and were much shorter than those of the check plants. The plants produced pods but no seeds, and as compared with the plants grown in the solution lacking potassium, the yield per plant was three times as great, giving an average of 1.05 gm. of dry matter per plant as against a corresponding yield of only 0.374 gm. from the plants grown in the potassium-free solution. It thus appears that sodium may replace potassium during the early stages of growth only, perhaps to the flowering stage or a little before, since no physiological disturbances were apparent in these plants which compared very well with the plants from the check cultures during their early stage of growth.

In the absence of iron, chlorosis was not observed until after the plants had been 10 days in solution. After this period the mature leaves turned yellow and died. The roots, however, appeared normal, both in size and color, throughout the growth period. Although new leaves and blossoms appeared, no pods developed. The average yield was the same as that produced by the solution lacking phosphorus, giving an average of 1.40 gm. per plant.

The intervals at the beginning of the growth period during which the plants in the different solutions appeared to grow normally and before any injurious effects were observed, varied greatly. The plants showed the injurious effects of the lack of calcium in 3 days, as did also those grown in the solution lacking potassium and in the nitrogen-free solution. In the absence of phosphorus or sulfur, the plants appeared to grow normally during the first 2 weeks, while only 7 days elapsed before injury was observed in the plants from the magnesium-free solution.

As previously stated, the plants from the different solutions were analyzed for nitrogen, calcium, magnesium, and ash content. The numerical data relating to these analyses are presented in table 3.

It is obvious that plants can make considerable growth by using as their only source of the mineral elements those stored as reserve in the seed. In order to determine, therefore, the amounts of the various mineral elements taken up by the plants from the solutions in which they were grown, it was

necessary to deduct from the total amount of each element found by analysis of the plant material, the amount of the corresponding element found by analysis of the seed. Duplicate samples of 100 seeds were dried to constant weight and analyzed quantitatively for the elements here considered. The average values per seed thus obtained were used as the basis for all deductions made from the total amounts of the elements found in the plants. Table 3 is, therefore, divided into two vertical sections, the first section giving the total average percentage values of the elements found in the plants from

TABLE 3

Average composition of plants grown in check solution and in solutions lacking one essential element

CULTURE NUMBER	ELEMENT LACKING	TOTAL CONTENT				ABSORBED FROM SOLUTION			
		Ash	Ca	Mg	N	Ash	Ca	Mg	N
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1*	....	13.31	1.34	1.10	3.22	12.95	1.29	1.09	2.76
2	Ca	12.50	0.57	0.65	7.03	7.90	....	0.43	1.15
3	Mg	18.41	1.52	0.10	4.34	16.75	1.46	....	2.12
4	K	12.69	3.15	1.15	4.55	10.24	2.80	1.02	1.28
5	N	15.11	4.53	0.37	5.33	11.10	3.82	0.14	0.28
6	P	8.61	1.74	0.75	3.44	8.05	1.67	0.73	2.67
7	S	15.79	1.15	1.35	4.48	15.02	1.02	1.40	3.38
8	Fe	15.73	1.39	1.12	4.31	14.48	1.30	1.01	3.50
9†	K	16.08	2.74	1.82	4.28	15.31	2.63	1.78	2.12
Relative values									
1*	....	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2	Ca	0.94	0.43	0.50	2.14	0.61	....	0.40	0.41
3	Mg	1.40	1.16	0.09	1.34	1.29	1.18	....	0.77
4	K	0.95	2.35	1.00	1.41	0.79	2.26	0.93	0.46
5	N	1.06	3.30	1.65	1.06	0.86	3.08	0.13	0.10
6	P	0.65	1.30	0.68	1.07	0.62	1.34	0.67	0.97
7	S	1.18	0.86	1.22	1.39	1.16	0.82	1.28	1.22
8	Fe	1.17	1.03	1.00	1.34	1.12	1.05	0.93	1.26
9†	K	1.21	2.04	1.65	1.36	1.18	1.05	0.93	1.26

* Check.
† Na replacing K.

series I and II. The second vertical section gives these total percentage values after deducting the corresponding calculated values for the seeds. The data of this section, therefore, represent the percentage of the elements actually absorbed from the solutions.

Since all analyses for each of the two series were carried out in duplicate, each value given in the table represents the average of at least four determinations. Some of these values represent averages of more than four determinations. The table is also divided into two horizontal sections, the second

horizontal section giving the data from the first section in terms of the values for the check taken as unity.

Ash content of plants

It will be observed from table 3 that considerable variation occurs in the ash content of the plants from the several cultures, and it is of interest to note that the plants from some of the incomplete culture solutions, notably those lacking magnesium, sulfur and iron, absorbed from solution considerably higher total percentages of ash constituents that did the plants grown in the complete culture solution (check). The plants from the solution in which sodium was substituted for potassium also absorbed a higher percentage of minerals from solution than did the check plants. On the other hand, the plants grown in the solutions lacking calcium, phosphorus, potassium, and nitrogen, showed much lower total relative rates of mineral absorption from solution than did the check plants, the lowest rate being shown for the plants grown in the solution lacking calcium. These had only a very slightly lower rate than did the plants in the solution lacking phosphorus, the percentages of ash absorbed being 7.90 and 8.05 respectively, as compared with 12.95 for the check. There appears, however, to be no definite relation between the mineral content of the plants and the yield of dry plant material, as is clearly brought out by figure 1.

Calcium, magnesium, and nitrogen content of plants

A survey of the data in the second vertical section of table 3 brings out the interesting fact that the percentage of calcium absorbed by the plants from the several incomplete culture solutions is, with a single exception, higher than that absorbed from the complete culture solution used as a check. The exception noted refers to the plants grown in the solution without sulfur, although attention must be called to the fact that this solution was not absolutely lacking in this element, since a trace of it was added to the solution in the form of ferrous sulfate, which was supplied in exceedingly minute quantity as a source of iron for the plants. The variations of relative calcium absorption from the several solutions are quite pronounced, ranging in value from 18 per cent below that of the check plants, indicated for the culture lacking sulfur, to 208 per cent above, indicated for the culture without nitrogen. It thus appears that the absence from the culture solutions here employed of any one of the essential ions except the SO_4 ion, brings about an acceleration in rate of calcium absorption. These higher rates of relative absorption are quite pronounced in the plants grown in the solutions lacking magnesium, potassium, nitrogen, and phosphorus, as well as in the plants from the solution in which sodium was substituted for potassium. This may not be significant, however, with respect to the plants grown in the solution without iron, since the relative amount of calcium absorbed by the plants

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from this solution is only 5 per cent higher than that shown for the check plants. Whether or not a deficiency of any one of these elements without its complete absence may cause a corresponding acceleration in the rate of calcium absorption, has not been determined.
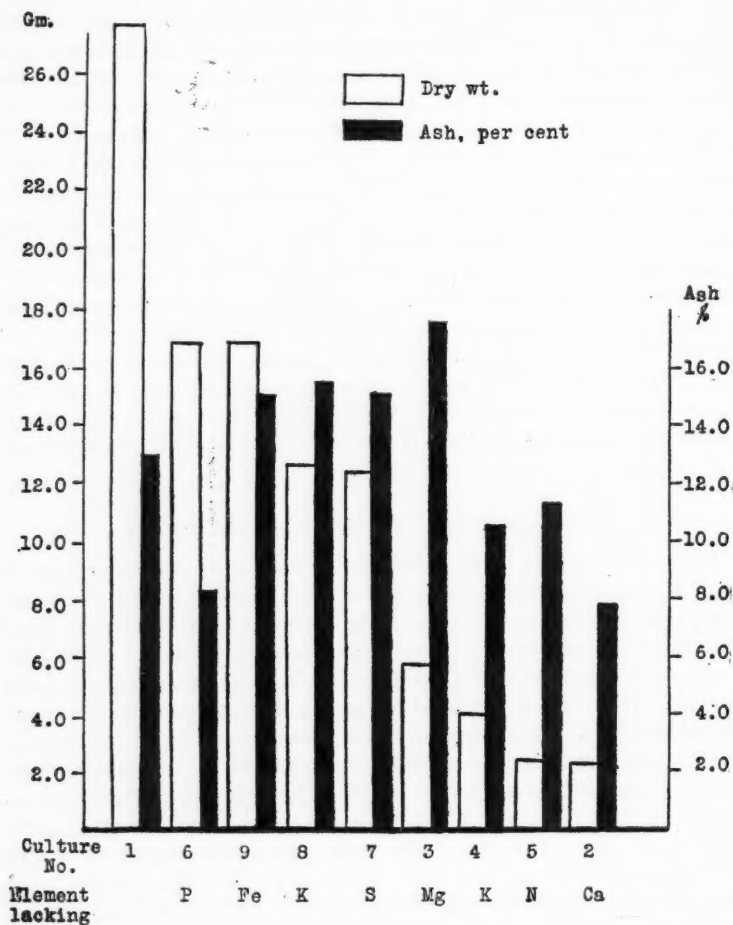
Fig. 1. Total Dry Weight and Ash Percentage of the Plants Grown in the Different Culture Solutions

It will be observed that the percentage of magnesium absorbed by the plants from the incomplete solutions, is low where calcium absorption is high and high where calcium absorption is low, relative to the percentages of

these elements absorbed by the check plants.   This relation is quite definite
for all cultures except that in which sodium is substituted for potassium.
Here the relative absorption of magnesium was considerably higher than that
shown for the check plants.   It is thus evident that the absence from the
culture solution here used of any one of the essential ions has just the oppo-
site effect upon the absorption of magnesium by the plants from that which
it has upon the absorption of calcium.

The lowest percentage of magnesium absorption is shown for the plants
grown in the solution without nitrogen, and the highest for the plants in the
solution lacking sulfur (leaving out of consideration the culture in
which sodium was substituted for potassium).   It is interesting to note that
the plants in these solutions showed, respectively, the highest and lowest
relative rates of calcium absorption.   The relative rate of magnesium absorp-
tion by the plants from the solution without nitrogen was very low, being only
13 per cent of that shown for the check plants, while plants grown in the
solution lacking calcium and those in the solution without phosphorus also
showed very low rates, being respectively, 40 per cent and 67 per cent of that
of the check plants.

As may be seen from table 3, the relation between the percentage values
referring to nitrogen and calcium absorption is the same as that between
magnesium and calcium.   That is, low rates of nitrogen absorption by the
plants in the incomplete solutions correspond to high rates of calcium ab-
sorption, relative to the absorption rates of these elements by the plants in
the check solution.   This relation is also definite for all the cultures except
that lacking iron.   Thus the absence from the culture medium of any one of
the essential elements which tends to accelerate the rate of calcium absorption
by the plants, at the same time retards the rates of absorption of both mag-
nesium and nitrogen.   In other words, a high calcium content of the plants
grown in these incomplete solutions corresponds to a low content of both
magnesium and nitrogen, and a low calcium content corresponds to high
magnesium and nitrogen content relative to the check considered as unity.

Finally, attention is called to the fact that the plants grown in the solu-
tion which was supposed to be without nitrogen, in some unknown way accu-
mulated a small percentage (0.28 per cent as shown in the table) of this ele-
ment above that which can be accounted for in the seeds from which the
plants were grown.   Although this small excess nitrogen is not accounted
for, several sources may be suggested.   It is possible that traces of it in the
form of ammonia may have been dissolved in the culture solutions from the
atmosphere, since no particular precautions were taken to prevent this.   It is
possible also that in the process of distillation, the water used in the prepara-
tion of the culture solutions may not have been completely freed of ammonia,
although all the water so used was obtained from a Barnsted still in good
condition.

SUMMARY

Investigations were carried out to determine the relation of each of the essential mineral elements to the composition and physiological characteristics of soybean plants grown to maturity in culture solutions. Plants harvested from eight solutions, each of which lacked one of the essential elements, and from Shive's R_5C_2 solution used as check, were analyzed for calcium, magnesium, nitrogen, and total ash content. In one of the eight solutions potassium was replaced by equivalent amounts of sodium. The nature of the injury sustained and the appearance of the plants are described.

Pathological conditions due to the lack of any one element, appeared first and most pronounced in plants grown in the calcium-free solution, followed in order in the plants grown in solutions without nitrogen, potassium, magnesium, sulfur, iron, and phosphorus. Sodium appears to replace potassium only to the time of blossoming. The plants from the solutions lacking phosphorus produced non-viable seeds.

Pronounced variations in the ash content of the different plants were observed, ranging from 8.61 per cent where phosphorus was lacking to 18.41 per cent in the absence of magnesium, as compared with 13.31 per cent of the check plants.

The plants grown in the incomplete culture solutions, with the exception of those grown in solutions lacking sulfur, always absorbed higher percentages of calcium and lower percentages of both nitrogen and magnesium than did the check plants. High calcium content in these plants corresponds to low nitrogen and magnesium content, and low calcium content corresponds to high nitrogen and magnesium content, relative to the check plants considered as unity.

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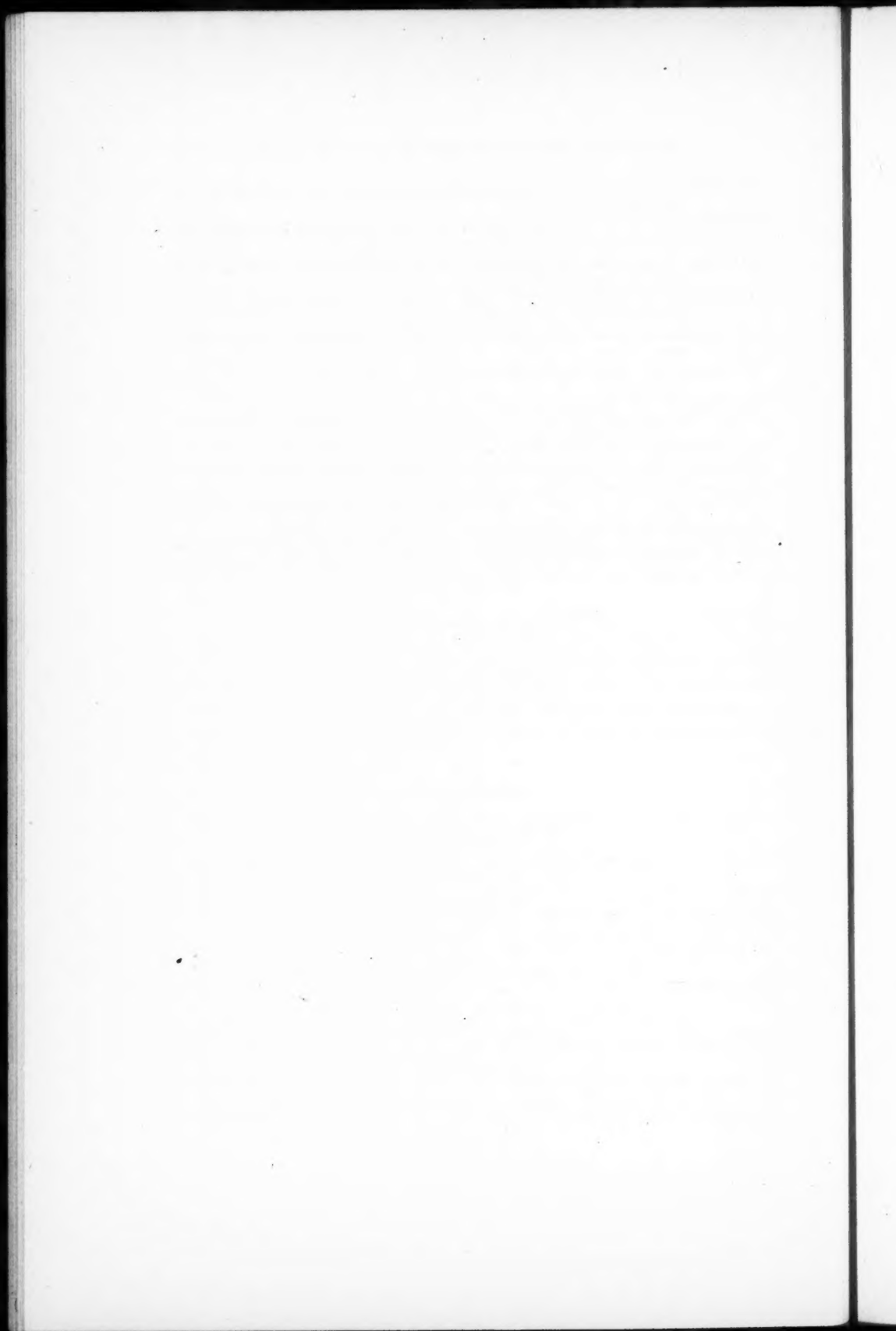
INFLUENCE OF WATER AND SALT SOLUTION UPON ABSORPTION AND GERMINATION OF SEEDS[1]

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INTRODUCTION

The influence of chemical reagents upon the germination of seeds has been studied by many investigators with special reference to the questions: (*a*) which chemicals can be used to stimulate germination, (*b*) what strengths of the reagents can be used without injury, and (*c*) what is the effect of leaving salts in contact with seeds for a long period.

Since Von Humboldt, at the end of the eighteenth century, observed that old seeds soaked in chlorine water germinated better, many investigators have employed salt solutions of different strengths.

The factor of osmotic concentrations of the different salts used has generally been overlooked. That the osmotic concentrations of the salt solutions play an important rôle in absorption by seeds has been demonstrated by the author in an earlier study (16), where the different salt solutions were compared with each other and with distilled water. The distilled water comparison is not ideal, but it is necessary. Frequently seeds and seedlings are seriously injured by soaking the seeds in water; growth is sometimes retarded, or, as in the case of thick-coated seeds, stimulated. Absorption and germination of seeds in soils are not directly comparable with absorption and germination of seeds in contact with salt solutions, but the results obtained in the laboratory may be made useful to the study of the complex conditions existing in the soil. The osmotic concentrations of commercial salt solutions, which are brought into the soil in rather large quantities by drilling, vary; however, the concentrations usually change but slowly on account of the limited amounts of water available.

REVIEW OF LITERATURE

Many of the early investigators have tried to determine the limits within which seeds would germinate. Most work was done in so haphazard a way that no data is available for comparisons and is consequently of little value.

The literature of later years on the subject can be divided into two parts;

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that which treats of reagents which stimulate to a certain extent and then become harmful, and studies of the effect of salts in contact with seeds during the germination process to determine the fertilizing value.

Stimulating effects

Vandervelde (21), studying the influence of chemicals upon the germination of seeds, comes to the conclusion that the germination power and germination energy decrease with the increase of concentration, but that germination becomes greater after the concentrations have reached a certain point.

Buffum (2), in his alkali studies, found that very small amounts of Na_2SO_4, NaCl, $MgSO_4$, and Na_2CO_3 have a beneficial effect upon germination and growth. The conclusion drawn is that the retarding effect of a salt on the germination of seeds is in direct proportion to its osmotic pressure, except where other factors enter in, such as the caustic effect of Na_2CO_3, or where solutions are very dilute. These conclusions are confirmed in our studies for higher concentrations.

Schlek (18) found that the influence of the concentrations of fertilizer salts was no greater than that of pure water. Promsey (14), while studying the rôle of acids in germination, found that weak acidity in the salt solutions used was favorable to germination, and that salts of the stronger acids induced turgescence but did not decrease the weight. Lesage (10, 11, 12) observed that germination decreased as the solutions were made more dilute, but that when a certain point was reached germination increased again. He was able to construct a curve which showed this.

Investigations made by Crochetelle (5) on the influence of manganese sulfate show that this salt favors germination in solutions of 5:10,000. The effect of soaking seeds in water was studied by Kidd and West (9), who came to the conclusion that at all temperatures, peas and beans are injured by being soaked in an excess of water; the number and vigor of the plants produced are diminished. This injurious effect was more marked in the lower temperatures (5° to 10°C.) than in the medium temperatures (15° to 20°C.), the amount of injury again increasing in the higher temperatures, as is shown by the curve of the number of plants produced from seeds at different temperatures. This curve rises to an optimum in the region of 15° to 20°C., and then falls.

Effects of salt solutions in constant contact with seeds

In determining whether any substance be favorable or injurious to plant growth, the presence of any chemical agents aside from the substance to be tested is a complication to be avoided, or at least controlled, since there may be either a direct relation between the different substances in the substratum or an indirect influence exerted through changes induced in the plant. When seeds are in constant contact with the salts used, particular care must be taken. Claudel and Crochetelle (3), studying the influence on germination

of certain substances used as fertilizers, found that immediate contact had an injurious effect upon germination.   Alkaline substances with Ca or K as a base, favor germination of many kinds of seeds, especially legumes.   The conclusion was that alkaline substances are beneficial in proportion as they neutralize acids produced by germination.   Stewart (20) concludes, however, that alkalines are most injurious to legumes, so that cereals would prove a much surer crop than legumes on alkali soils.   According to Hicks (8), 1 per cent KCl and $NaNO_3$ are very detrimental to germination, whether applied direct or mixed with soil.   Fertilizers with HPO_4 or Ca were less injurious.

From experiments with 24 kinds of salts, applied at the rate of 10 gm. per 115 kgm. soil, Rusche (17) concludes that KCl stimulated germination (the germination power was lower) for wheat, peas, beans and rape; but clover, alfalfa, and lupine were harmed.   He found that: in all cases, $MgSO_4$ had a very good influence; K_2CO_3, especially, stimulated the germinating power of the seeds; and NaCl was very good for lupine, barley, and rape, but was harmful to clover and alfalfa.   Most of the conclusions of this worker are contradictory to the experience of other investigators.   Bokorny (1) left the seeds in the salt solutions during the whole germinating period.   He concludes that none of the salts used has a beneficial effect.   The seeds in water germinated as well as in any solution.   All salts were more or less harmful in solutions of 1 per cent, except KH_2PO_4 which was injurious in 2 per cent solutions.   Shive (19) germinated beans and corn from 48 to 72 hours in sand at room temperature, using salt solutions of an osmotic concentration of from 0.5 to 8 atmospheres.   His conclusion was that, while germination was not prevented by the higher salt concentrations, it was considerably retarded; that is, the higher the concentration of the solution added to the sand culture, the greater was the time required for germination to take place.   While germination was not actually prevented by the highest concentrations employed, injury to the root tips occurred after germination had taken place, even in concentrations as low as 2 atmospheres of possible osmotic pressure.   In our study we found that root tips were injured in still lower concentrations, as well as in distilled water.

Maguenne and Demoussy (13) conducted experiments, at room temperatures, on the influence of mineral salts upon the germination of peas.   They used only 10 seeds in 40 gm. of sand and 10 cc. of liquid.   The length of roots after 24 hours soaking and 6 days germination was recorded in millimeters. In the higher concentrations NaCl was beneficial, $MgSO_4$ not harmful, and $CaSO_4$ had a decidedly stimulating effect.

The effects of salt-treated soils on the absorption by seeds were studied by Gericke (7).   He came to the conclusion that small applications of Na_2CO_3 to soils increased the weight of beans, barley and corn through increased absorption by the seeds.   Coe (4) states that direct contact of fertilizers with seed retards and inhibits their germination.   Under greenhouse conditions $NaNO_3$ is more toxic to corn than ammo-phos or $(NH_4)_2SO_4$, NH_3NO_3 less toxic than $NaNO_3$, and KCl more toxic than K_2SO_4.

No such extensive data have been presented on the influence of salts upon the structure of plants. In the work reported below, special attention is not paid to the peculiarities which sometimes occurred in the shape of the roots, the odd ways of germination, nor to the particular length of secondary roots and root hairs; but in a few instances where the phenomena were very striking they were recorded.

The fundamental investigations published in a series of articles by Dassonville (6), might be mentioned here. He reports that when nitrates were left out of the Knop's solution the root system of rye was greatly extended. $MgSO_4$ shortened the roots of most of the seedlings and injured lupine and ricinus, particularly. Anatomic changes that occurred were great cell thickenings and enlarging of the parenchyma cells.

SCOPE OF THE INVESTIGATION

The foregoing review indicates the contradictory nature of the results already obtained. The factor of the osmotic concentrations of salt solutions has been very little emphasized. This has been more fully discussed in an earlier paper by the author (16) on the absorption by seeds in salt solutions with definite osmotic concentrations. Two points, especially, were open to further study:

1. The stimulating effect on seeds soaked in salt solutions with known osmotic concentrations.
2. The injurious effect upon the germination of seeds in salt solutions of definite osmotic concentrations, when the seeds were in continuous contact with the solutions.

EXPERIMENTAL WORK

Analyzed salts of high quality were used in all experiments. The concentrations of the solutions were calculated and determined by the freezing point method. They varied by increments of one atmosphere, beginning with 1 atmosphere and increasing to 7 atmospheres. The range of concentrations, however, was from 0.001 to 7 atmospheres. The salts used were: $MgSO_4$, $NaNO_3$, $Ca(NO_3)_2$, NaCl, K_2CO_3, KCl, KH_2PO_4, KNO_3, and Shive's three-salt solutions consisting of KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ in partial osmotic concentrations of 5–2–3, respectively. Stock solutions of 0.5 molecular were made, which lasted throughout the investigation. The desired concentrations were subsequently prepared by dilution and the osmotic strength was determined.

The seeds used were: white lupine, watermelon, Canada field peas, buckwheat, soybeans, wheat, corn, beans, alfalfa, and dwarf rape. For each series 50 seeds were used except for rape and alfalfa where 100 seeds were employed. The total number of seeds compared for each salt in each table was, therefore, 650 for the larger seeds, and 1300 for the smaller. All seeds were soaked for 15 hours in salt solutions of sufficient amounts to cover them after the seeds

were swollen.   With each such series was a series of seeds which were soaked in an equal quantity of distilled water and used for comparison.   Still another series was added, where the seeds were germinated without previous soaking (a "dry" series), in order to determine the germination of the seeds under more "natural" conditions.

In that part of the study where the object was to determine the stimulating effect of the salt solutions used, the seeds were, after the period of soaking, quickly rinsed by shaking for a few seconds in distilled water.   The seeds were then placed between double folded beds of filter paper.   This method is not entirely satifactory on account of evaporation, but it has great advantages over sand beds.   In sand beds, particles of sand cling to the roots and root hairs and are very difficult to remove without breaking the roots, thereby causing the loss of some parts, or necessitating the weighing of small quantities of sand together with the seedlings.   The amounts of solution and water necessary were determined as nearly as possible in preliminary experiments.   The beds were uniformly moistened twice a day, and possible errors would be much the same for all series.   These seedbeds were kept in an incubator at a temperature of 27° or 28°C. for the length of time stated in the tables.   All experiments were made in duplicate.   The essential data are given in the tables, where the percentage absorption is based on the weight of the seeds, and the length of the roots and shoots are compared.   The percentage of germination and the percentage of roots injured are also given.   The tips being brown or black was taken as an indication of injury to the roots.

EXPERIMENTAL RESULTS

Influence of distilled water

In the experiments from which data are presented in table 1, an effort was made to determine the influence of absorption upon the germination of the seeds.   It is evident that the soaking of seeds in distilled water is harmful. In nearly all cases the seeds which were not subjected to water or salt solution treatments germinated better than the soaked seeds.   The roots of the dry seeds were generally shorter after the periods given, but they soon acquired the same relative length as the roots of the soaked seeds.   This fact was brought out when these seeds were allowed to germinate for a period of from six to seven days.   In several cases, lupine, when not soaked, had relatively shorter roots after such a time; but these appeared to be thicker, although less branched.   The data obtained, however, are not conclusive, and for this reason are not included.

Kidd and West (9) concluded that the soaking of peas and beans in an excess of water is harmful.   This seems to be true for other seeds as well, at the temperature used in our studies.   Buckwheat was badly injured.   In general no marked difference in the percentage germination occurred between the seeds

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TABLE 1

Influence of soaking of seeds in single salt solutions upon germination at a constant temperature of 27–28°C.

Seeds imbibed for 15 hours

ATMOSPHERES	PEAS GERMINATED FOR 100 HOURS IN NaNO₃				WHITE LUPINE GERMINATED FOR 7 DAYS IN NaNO₃				BUCKWHEAT GERMINATED FOR 135 HOURS IN NaNO₃			
	Absorbed	Germination	Average length of roots	Roots injured	Absorbed	Germination	Average length of roots	Roots injured	Absorbed	Germination	Average length of roots	Roots injured
	per cent	per cent	mm.	per cent	per cent	per cent	mm.	per cent	per cent	per cent	mm.	per cent
7	85	36	17	33	137	10	16	80	43	32	15	28
6	87	52	19	26	139	12	12	83	43	34	21	26
5	88	40	26	19	137	20	31	70	43	36	21	20
4	88	64	27	12	138	26	27	85	45	36	26	14
3	88	56	27	11	142	44	26	60	45	26	19	12
2	89	56	33	7	144	78	31	28	46	24	15	8
1	90	76	29	3	146	80	35	35	47	38	20	6
0.5	92	66	31	7	142	68	34	16	48	34	21	7
0.1	92	78	35	8	146	64	34	9	48	30	18	7
0.01	91	—	—	—	148	66	43	9	48	26	24	7
0.001	91	64	28	7	153	66	47	9	48	30	27	7
H₂O	88	62	31	5	149	66	43	6	50	34	10	4
Dry		96	24	0		82	9	0		80	39	0
	WHITE LUPINE GERMINATED FOR 72 HOURS IN Ca(NO₃)₂				PEAS GERMINATED FOR 72 HOURS IN Ca(NO₃)₂				WATERMELON GERMINATED FOR 120 HOURS IN Ca(NO₃)₂			
7	90	10	8	100	126	12	5	100	69	54	41	No
6	91	14	17	57	125	12	7	66	68	76	40	record
5	93	12	18	50	129	10	10	60	71	68	47	
4	92	24	22	66	131	22	13	48	71	74	46	
3	92	20	30	40	133	52	14	46	71	76	45	
2	93	26	27	40	135	68	18	44	72	72	48	
1	94	30	24	60	137	76	27	28	69	76	72	
0.5	98	40	17	80	140	64	32	16	73	78	67	
0.1	98	36	19	88	147	64	26	10	79	78	72	
0.01	98	34	18	76	149	64	23	9	81	74	63	
0.001	98	36	20	83	148	50	18	8	81	66	71	
H₂O	98	54	18	4	148	58	31	10	80	60	66	
Dry		76	15	0		82	17	0		82	48	
	SOYBEANS GERMINATED FOR 96 HOURS IN NaCl				WHEAT GERMINATED FOR 100 HOURS IN NaCl				WATERMELON GERMINATED FOR 125 HOURS IN NaCl			
7	119	72	47	44	39	96	17*	10	65	64	58	14
6	119	76	70	19	39	97	16	8	68	72	59	3
5	118	80	77	20	40	96	16	6	73	72	62	2
4	120	90	84	16	41	98	16	4	73	74	60	4
3	121	90	92	15	41	94	17	3	73	74	59	1

* Length of stems.

TABLE 1—*Continued*

ATMOSPHERES	SOYBEANS GERMINATED FOR 96 HOURS IN NaCl				WHEAT GERMINATED FOR 100 HOURS IN NaCl				WATERMELON GERMINATED FOR 125 HOURS IN NaCl			
	Absorbed	Germination	Average length of roots	Roots injured	Absorbed	Germination	Average length of roots	Roots injured	Absorbed	Germination	Average length of roots	Roots injured
	per cent	per cent	mm.	per cent	per cent	per cent	mm.	per cent	per cent	per cent	mm.	per cent
2	118	88	99	11	41	92	19	1	78	70	60	0
1	121	89	99	13	43	98	19	0	80	72	60	0
0.5	128	90	109	9	44	98	19	0	82	80	64	1
0.1	129	84	109	16	44	100	19	0	80	68	65	2
0.01	130	80	106	12	43	96	20	0	82	68	68	1
0.001	126	92	102	7	43	96	20	0	80	72	64	0
H₂O	132	88	101	7	41	98	17	0	81	58	20	0
Dry		98	45	0		96	9	0		84	39	0

ATMOSPHERES	ALFALFA GERMINATED FOR 48 HOURS IN NaCl				RAPE GERMINATED FOR 96 HOURS IN NaCl				WHITE LUPINE GERMINATED FOR 5 DAYS IN K₂CO₃			
7	119	86	15	8	58	84	14	4	148	0	0	0
6	122	83	20	5	58	86	14	4	149	0	0	0
5	123	85	22	4	56	86	14	3	150	0	0	0
4	124	88	20	3	57	87	15	5	147	0	0	0
3	125	88	23	4	57	87	15	3	146	2	10	100
2	128	88	23	3	57	94	16	1	145	6	20	100
1	129	90	24	2	63	92	16	4	143	44	28	100
0.5	128	87	22	2	58	88	16	3	143	72	29	52
0.1	145	86	23	1	58	86	14	2	142	74	57	41
0.01	146	90	23	0	58	90	17	0	144	84	60	19
0.001	147	86	20	0	58	89	14	0	146	88	55	20
H₂O	146	84	21	0	61	97	14	0	145	84	38	7
Dry		87	13	0		92	15	0		72	8	0

ATMOSPHERES	SOYBEANS GERMINATED FOR 72 HOURS IN KCl				WHITE LUPINE GERMINATED FOR 100 HOURS IN MgSO₄				WATERMELON GERMINATED FOR 96 HOURS IN MgSO₄			
7	95	94	41	11	123	14	7	43	75	94	5	42
6	98	100	55	9	126	22	6	37	77	92	51	31
5	102	96	58	4	126	18	9	39	78	100	54	27
4	103	98	63	3	132	20	10	25	81	98	58	22
3	107	96	66	2	134	18	15	26	81	100	60	19
2	109	96	65	0	137	48	20	25	81	99	76	14
1	117	98	67	0	141	76	23	20	84	100	83	10
0.5	110	98	68	0	145	88	24	9	84	82	93	7
0.1	112	100	73	0	147	72	29	0	85	78	93	0
0.01	110	96	74	0	149	66	27	0	93	68	93	0
0.001	110	94	67	0	144	80	29	0	88	80	88	0
H₂O	111	98	67	0	148	86	32	6	90	72	77	3
Dry		98	13	0		94	18	0		84	43	0

soaked in distilled water and those in the salt solutions of very low concentration, but usually root length was greater in low salt concentration than in distilled water.

Influence of salt concentration

It is interesting to note that lupine roots were relatively much less injured in the $MgSO_4$ series than in the other salt solutions. No injury to the root tips occurred in the extremely dilute salt solutions. The percentage of injury in the stronger concentrations, however, was markedly greater than in distilled water. With the relative decrease in absorption, germination also decreased. The solution of 3 atmospheres seemed almost as harmful as the higher concentrations. The relative root-length decreased in the same way. Watermelon was, on the contrary, decidedly stimulated in $MgSO_4$ solution as compared with distilled water and the extremely dilute solutions. Even in the solution of 7 atmospheres, where the absorption was 7 per cent less, the germination was higher for the soaked, than for the dry seeds. The average root-length for the seeds in the weaker solutions was much greater, being even 20 per cent more in 0.5 atmosphere. In from 3 to 6 atmospheres the relative root-length decreased, and reached its lowest point in the solutions of 7 atmospheres.

The absorption by peas in $NaNO_3$ solution was greater in all the weaker solutions than in distilled water, while the absorption in the higher concentrations was the same or only slightly less. The germination also, in most cases, was greater in the weaker $NaNO_3$ concentrations, but was decidedly less for all seeds soaked in these salt solutions or water, than for dry seeds. White lupine soaked in $NaNO_3$ solution was under observation for 7 days. It seemed remarkable that seeds in 1 and 2 atmospheres germinated much better than those in either the lower or the higher concentrations. They also produced longer roots, and the injury was comparatively much less than in the higher concentrations. None of the seedlings from the dry seeds showed injury after this time, but the root growth was much less than for the soaked seeds.

Calcium nitrate had a detrimental effect upon the germination and root length of nearly all seeds used. The absorption, also, decreased towards the stronger concentrations. Lupine seems to be an extremely sensitive seed. No cause could be found to explain why, in two series with distilled water conducted side by side, the seeds of one series should germinate better than those of the other. In our experiments it was repeatedly observed that these seeds would sometimes germinate as well in distilled water as the unsoaked seeds, while the germination of other seeds, under apparently the same circumstances, was far less than that of the dry seeds. In general, however, the soaking in distilled water, as well as in salt solutions, was harmful to lupine. Calcium nitrate appeared to be harmful to the roots; this was particularly noticeable in the case of peas. This salt has a tendency to rot the peas quickly. In all solutions there was a larger percentage which did not germinate, but rotted soon. For the series in the stronger concentrations, this percentage was

about 90. This is contrary to the action of $NaNO_3$ which seemed to preserve buckwheat, as is reported elsewhere in this paper.

Soybeans were injured in the strongest solutions of NaCl; the injury in the weaker solutions did not differ much from the injury in water. Although the absorption by soybeans, watermelon, and alfalfa was less in NaCl, the germination of these seeds was, in many instances, better, and the root-growth was stimulated, this stimulation being very marked for watermelon. The germination of this series was better, as compared with that in distilled water, and the roots, in most instances, reached a length three times as great as that of the seedlings from the seeds which had been previously soaked in water. A number of seeds soaked in the NaCl solutions produced extremely long roots, as compared with the seeds in distilled water and the dry seeds. This was so noticeable that the roots were measured and classified into: roots longer than 100 mm., lengths between 100 and 50 mm., those between 50 and 25 mm., and those which made only a slight growth. Of the seeds soaked in a solution of 0.5 atmospheres, 12 had roots longer than 100 mm., but only 6 of the seeds soaked in distilled water and the unsoaked seeds had such a length. The number of seedlings with extremely long roots decreased gradually with the increase in the concentrations of the solutions. The injured seeds counted were all among the ones which made only an attempt to grow. The same effect could be noticed, though to a less extent, for wheat and soybeans. The same phenomenon occurred in solutions of $NaNO_3$ and KCl.

The roots of rape were stimulated in solutions of NaCl of from 0.01 to 4 atmospheres; KCl had much the same influence as NaCl. The absorption was less with the increase in concentration, but the germination remained the same. The growth of the roots was stimulated in the weaker solutions, but the two strongest concentrations were harmful.

The influence of K_2CO_3 upon germination and root growth was very decided. The absorption in the stronger concentrations was greater, due undoubtedly to the attack on the seed coats by this salt. The most striking data are included in this table. White lupine did not germinate at all in solutions of from 4 to 7 atmospheres; only a few seeds germinated in solutions of 2 and 3 atmospheres, and all roots were injured. The germination in weak solutions was as good as in distilled water and the root-growth much better, but injury increased with the increase of the salt concentrations.

Influence of salt solutions in constant contact with seeds

To obtain an indication of the influence upon germination of salt solutions kept in contact with seeds, root lengths are compared. In order to determine the root length with more ease and accuracy, three large seeds, flint corn, beans, and watermelon, were chosen. The results are recorded in table 2. Osmotic salt concentrations ranging from 0.5 to 7 atmospheres were employed, with one control series in distilled water for every salt and seed. The seeds were not soaked but were placed dry in filter paper beds, which were kept moist

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TABLE 2

Influence of salt solutions upon absorption and germination of corn, beans and watermelon when seeds were kept moist with salt solutions continuously for 6 days

Experiments conducted at laboratory temperature of 18 to 20°C.

| ATMOSPHERE | Ca(NO₃)₂ Corn | | | Beans | | MgSO₄ Corn | | | Beans | | K₂CO₃ Corn | | | Beans | |
	Relative absorption	Relative length of roots	Relative length of shoots	Relative absorption	Relative length of roots	Relative absorption	Relative length of roots	Relative length of shoots	Relative absorption	Relative length of roots	Relative absorption	Relative length of roots	Relative length of shoots	Relative absorption	Relative length of roots
7	45	10	27	77	0	47	5	0	89	50	52	13	0	86	55
6	47	12	27.	80	23	47	17	0	90	58	54	17	7	92	81
5	54	20	41	84	31	50	30	19	94	70	59	24	13	93	96
4	60	27	41	86	43	53	36	23	96	80	61	25	27	99	99
3	66	31	50	90	49	61	37	45	99	98	69	36	53	97	106
2	85	59	73	92	57	63	41	59	107	96	74	51	60	98	113
1	102	86	95	104	108	79	80	52	113	124	80	76	67	99	117
0.5	100	86	95	—	103	80	80	82	121	126	83	88	73	100	141
H₂O	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

| ATMOSPHERE | KH₂PO₄ Corn | | | Beans | | KCl Corn | | | Beans | | KNO₂ Corn | | Beans | |
	Relative absorption	Relative length of roots	Relative length of shoots	Relative absorption	Relative length of roots	Relative absorption	Relative length of roots	Relative length of shoots	Relative absorption	Relative length of roots	Relative absorption	Relative length of roots	Relative absorption	Relative length of roots
7	65	33	33	81	55	64	30	55	83	51	73	30		80	32
6	70	41	54	87	55	70	52	55	87	70	76	35		83	39
5	73	45	69	90	60	77	63	65	89	84	83	50		87	47
4	73	45	83	93	60	83	83	74	93	87	85	52		91	65
3	89	79	85	93	61	86	87	83	100	95	85	50		92	71
2	96	94	118	96	67	92	97	93	101	99	91	68		100	111
1	99	111	125	102	75	93	97	93	102	101	94	75		101	112
0.5	111	128	130	103	92	98	99	93	107	130	102	98		104	114
H₂O	100	100	100	100	100	100	100	100	100	100	100	100		100	100

ATMOSPHERE	SHIVE'S 3-SALT SOLUTION Corn			Beans		Watermelon		KH₂PO₄ Watermelon		KCl Watermelon	
7	73	65	54	87	53	65	2	75	17	70	5
6	80	65	60	93	66	61	9	86	16	70	8
5	80	74	65	94	67	72	23	91	22	71	33
4	82	80	68	94	69	80	41	116	43	75	36
3	84	84	71	94	71	80	56	129	52	82	44
2	96	98	98	94	76	80	57	145	81	95	98
1	102	96	88	94	72	86	59	180	95	108	122
0.5	122	94	84	99	73	77	71	118	99	114	100
H₂O	100	100	100	100	100	100	100	100	100	100	100

with the salt solutions during the whole periods. The seedbeds were left standing at laboratory temperature in large glass dishes, covered to prevent evaporation.

The germination power of the corn was high, 100 per cent in almost all cases even in the beds kept moist with K_2CO_3. There was a marked difference in root- and top-growth for the different salt solutions. In nearly all cases the absorption of solution by corn decreased with the increase of concentration. Corn germinated for 70 hours in seedbeds to which $Ca(NO_3)_2$ solutions were added, gradually absorbed less as the concentrations increased. Root- and top-growth followed the same rule as absorption, though in a more pronounced manner, root-growth decreasing towards the higher concentrations at a faster rate than top-growth. In the solution of 7 atmospheres, the average root-growth was but little more than 10 per cent of the root-growth of seeds in distilled water, while the relative length of the tops was 27 as compared with 100 in distilled water. The seedlings in concentrations of 6 and 7 atmospheres of $MgSO_4$ did not produce any shoots. The root- and top-growth gradually decreased, starting from the weaker concentration. The seeds placed in K_2CO_3 germinated well, but all seeds were discolored to a yellowish brown. All root tips were injured in solutions of from 2 to 7 atmospheres. The length of the individual roots was very regular. This was not the case in the $Ca(NO_3)_2$ series.

The relatively short and long roots in KH_2PO_4 were noticeable. In this series the seeds placed in 0.5 atmosphere absorbed 11 per cent more than in distilled water, while the root- and top-growths were about 30 per cent more. This salt, in this particular concentration, seems to favor absorption and growth.

The germination in the KCl series was more or less irregular; however, absorption and root- and top-growth followed each other, decreasing with the increase of concentration. Corn germinated in beds to which Shive's three-salt solution was added, behaved in the same way as seeds in the single-salt solutions. Growth was no better than that of corn germinating in distilled water, but absorption was markedly greater in 0.5 atmosphere.

Beans, which were germinated for 48 hours between filter papers moistened with $Ca(NO_3)_2$, absorbed slightly more in the solution of 1 atmosphere, and produced longer roots than beans in beds moistened with distilled water. Absorption by beans in the $MgSO_4$ series was markedly increased in the lower concentrations. Decided increase in root-growth occurred in the solution of 1 atmosphere. There was less decrease in root-growth with the relative increase in concentration than was the case in the $Ca(NO_3)_2$ series. The ungerminated seeds in the latter series, however, were hard and looked as if they would germinate if given longer time. The seeds in the K_2CO_3 series were highly discolored to a dark red-brown. The root tips of the seedlings in concentrations of from 2 to 7 atmospheres were all very much injured, some of them decomposed. Root-growth of beans was stimulated in the lower concentration of KCl and KNO_3, but much less in the KH_2PO_4 series and in Shive's three-salt solution. The best in the two latter solutions reached a root length only three-fourths as great as those in the control in distilled water.

The series with watermelon were not all conducted at the same time. Those in the first series, which were with KH_2PO_4, were germinated in August, and those in the other series in the late fall. The difference in the time, and consequently in the temperature, may partly explain the difference in root-growth. The total germination in distilled water was the same for all series, but the root-growths in distilled water for the KCl and Shive's solution series show a
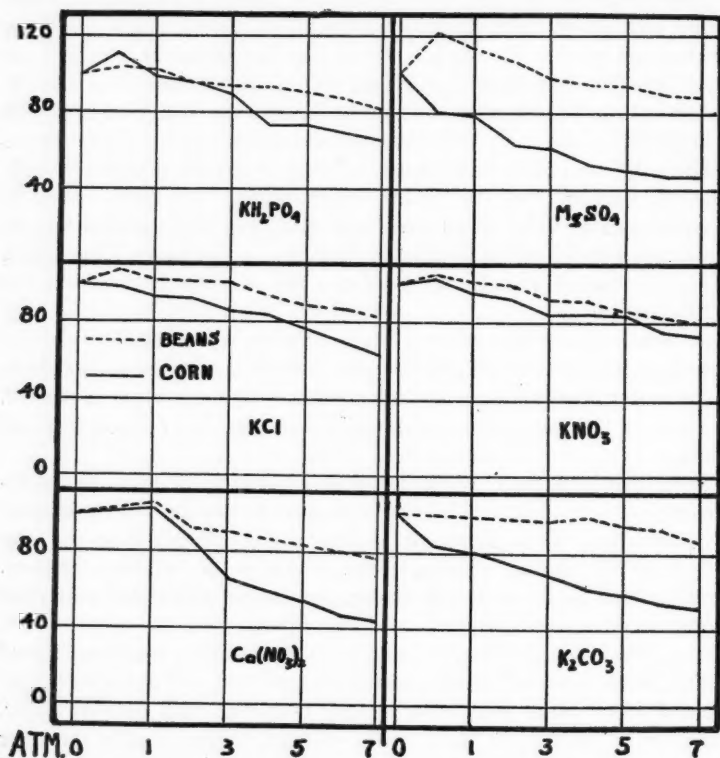
FIG. 1. RELATION BETWEEN RELATIVE ABSORPTION OF BEANS AND CORN IN SALT SOLUTIONS OF DIFFERENT OSMOTIC CONCENTRATIONS AS COMPARED WITH ABSORPTION IN DISTILLED WATER

0 = distilled water

large decrease, although the time of germination for the latter was about the the same as for the KH_2PO_4 series. This holds true for the whole series in both cases, so that the seeds germinated in beds with salt solutions can be compared with the seeds germinated in beds which were moistened with distilled water. The absorption of KH_2PO_4 was considerably greater in solutions of from 0.5 to 4 atmospheres, but no increase in root-growth occurred. In

fact, in all cases the root-growth was better in distilled water than in the solutions, with only one exception, in the KCl series of 1 atmosphere.

In nearly all cases, salt solutions in constant contact with corn, beans and watermelon exercised a harmful influence upon germination, as indicated by root- and top-growth.
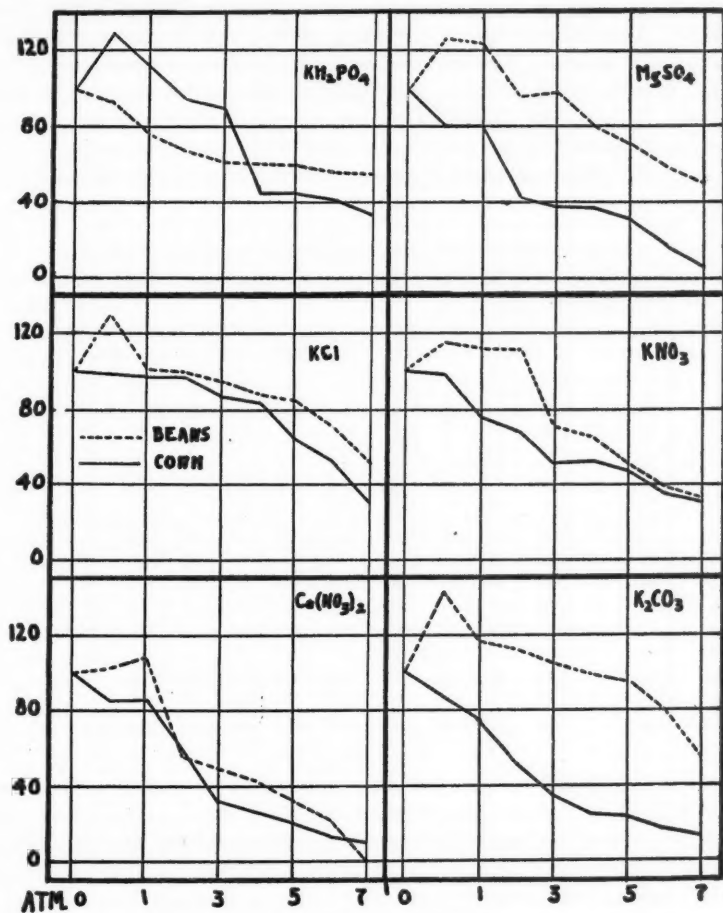
FIG. 2. RELATION BETWEEN RELATIVE ROOT-LENGTHS OF BEANS AND CORN

Seeds previously soaked for 15 hours in salt solutions of different osmotic concentrations. 0 = distilled water.

In figure 1 a comparison is made between absorption of beans and corn in different salt solutions. In practically every concentration of the different solutions the relative absorption of beans was greater than the relative absorp-

tion of corn. A comparison of the relative root-lengths of these two seeds (fig. 2) shows very similar results. As a rule beans are more readily stimulated by lower concentrations than corn.

The influence of salt solutions and root-growth of flint corn is shown in table 3. It is clear that the absorption from the different solutions is very similar, although the average absorption rates from the different salt solutions differ. A graphic comparison of relative absorption and relative root-length is given in figure 3. The rates of absorption and root-growth are proportionately retarded with the increase in osmotic concentration values of the solutions. If it is kept in mind that the comparison in this figure is made with distilled water it can be seen that some of the lower concentrations exert a marked stimulating effect upon root-growth, while others retarded root-

TABLE 3

Influence of salt solutions upon absorptions and root-growth of flint corn

Seeds soaked previously for 15 hours and thereafter kept moist with salt solutions for 48 hours. Results after 6 days.

ATMOSPHERES	Ca(NO₃)₂		MgSO₄		K₂CO₃		KH₂PO₄		KCl		KNO₃		SHIVE'S SOLUTION	
	Relative absorption	Relative length of roots	Relative absorption	Relative length of roots	Relative absorption	Relative length of roots	Relative absorption	Relative length of roots	Relative absorption	Relative length of roots	Relative absorption	Relative length of roots	Relative absorption	Relative length of roots
7	63	13	62	7	67	17	69	9	67	26	62	23	76	22
6	69	17	62	14	62	17	69	12	73	30	67	43	76	30
5	68	17	66	14	74	20	82	39	72	38	70	49	81	37
4	70	26	76	28	76	17	83	58	74	47	73	57	85	44
3	77	44	77	31	77	17	90	61	80	68	75	59	84	48
2	91	78	80	39	87	52	95	85	95	89	84	66	89	54
1	96	87	92	69	94	73	98	91	106	117	88	80	97	67
0.5	104	117	98	79	94	75	100	103	109	132	96	97	98	67
H₂O	100	100	100	100	100	100	100	100	100	100	100	100	100	100

growth considerably. The production of shoots as stimulated markedly by KH₂PO₄ in osmotic concentrations of 0.5, 1 and 2 atmospheres, while Shive's three-salt solution of 0.5 and 1 atmosphere produced decidedly shorter shoots than Shive's solution of 2 atmospheres. This solution as compared with the lower KH₂PO₄ concentrations had however a materially less stimulating effect upon shoot-growth (fig. 4).

Germination of corn in contact with salt solutions under constant temperature and duration

Plant cells have, in general, an osmotic pressure of from 5 to 11 atmospheres. Some writers give even much higher figures. The partial osmotic pressures
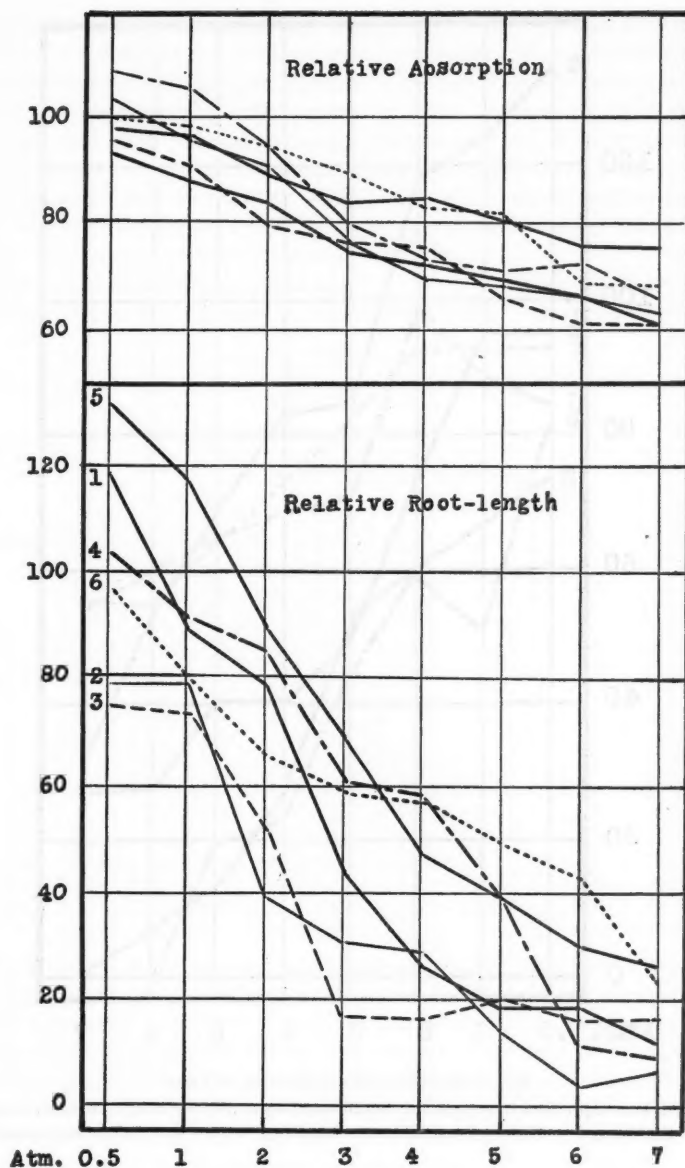
Fig. 3. RELATION BETWEEN RELATIVE ABSORPTION AND RELATIVE ROOT-LENGTH OF FLINT CORN

$1 = Ca(NO_3)_2; 2 = MgSO_4; 3 = K_2CO_3, 4 = KH_2PO_4, 5 = KCl; 6 = KNO_3$
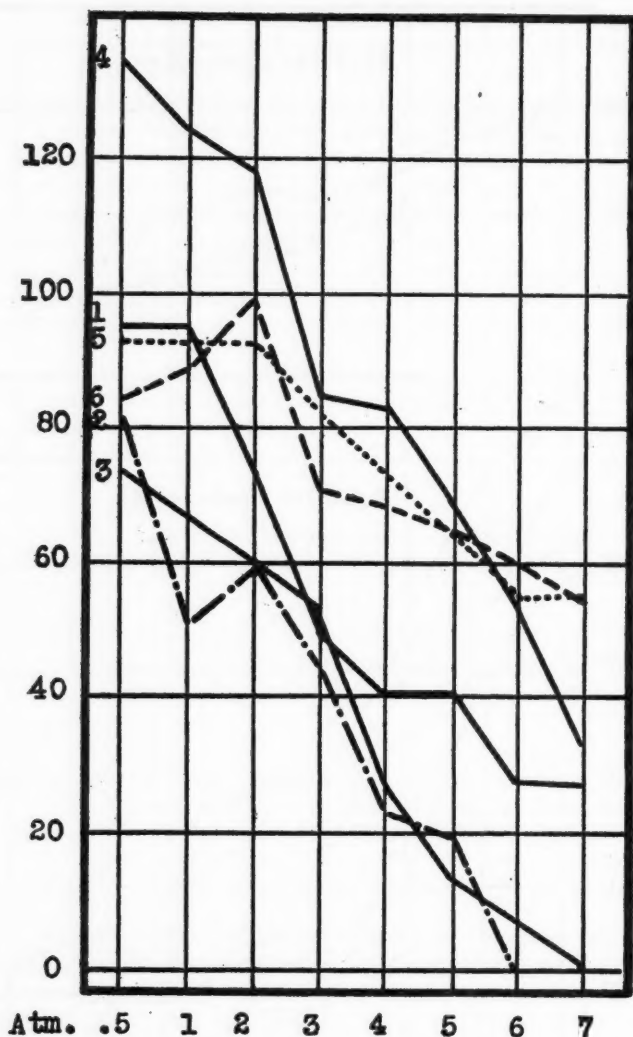
FIG. 4. RELATIVE SHOOT-GROWTH OF CORN

Seeds soaked previous to germination in salt solutions of different osmotic concentrations.
1 = Ca(NO₃)₂; 2 = MgSO₄; 3 = K₂CO₃; 4 = KH₂PO₄, 5 = KCl; 6 = Shive's R₅C₂ solution.

developed by some of the cell sap constituents when salt solutions are used, are still higher for many plants, and not infrequently reach 40, 60 and more atmospheres. Little is known, however, of the osmotic pressure or osmotic

concentrations in seeds.   That ions in solutions entering seeds produce great changes, and influence the activities, seems reasonable.   If all possible factors are not controlled, the question arises as to whether other influences than salt solutions were interfering with germination.   The temperature factor plays an important rôle.   According to van Rijsselberghe (15) the rate at which dissolved substances penetrate the protoplasm is dependent on temperature. Changes in temperature from day to night might therefore cause a decrease in the velocities at which the ions in solutions entered the seeds, and this in turn might influence the germination and root-growth.

The experiments conducted with flint corn, using a number of different salt solutions, subjected to a constant temperature and duration, as recorded in

TABLE 4

Influence of single-salt solutions upon the absorption and growth of corn at constant temperature of 27 to 28°C.

Results after 4 days

ATMOSPHERES	Ca(NO₃)₂			NaCl			MgSO₄			K₂CO₃		
	Relative absorption	Relative length of roots	Relative length of shoots	Relative absorption	Relative length of roots	Relative length of shoots	Relative absorption	Relative length of roots	Relative length of shoots	Relative absorption	Relative length of roots	Relative length of shoots
7	85	92	75	88	78	69	79	100	185	100	17	28
6	87	95	110	93	96	114	82	107	185	100	17	31
5	87	96	113	93	98	113	85	107	185	101	19	37
4	88	102	128	94	106	112	86	130	201	103	19	35
3	90	109	147	95	112	122	88	151	210	102	19	45
2	96	109	175	96	114	133	88	153	217	107	24	44
1	94	110	184	99	106	117	91	164	226	106	29	46
0.5	95	131	210	99	105	107	95	150	218	110	30	45
0.1	96	102	164	100	104	100	95	153	220	104	31	46
0.01	96	102	147	98	102	82	98	139	195	100	24	42
0.001	99	101	147	99	—	—	99	106	181	99	23	35
H₂O	100	100	100	100	100	100	100	100	100	100	100	100

table 4, show that there is no principal difference between the absorption under ordinary laboratory conditions and that when the temperature and length of time factors are controlled; absorption decreased with the increase in concentration.   A comparison of two of the MgSO₄ series with corn, one series kept at a constant temperature and the other at laboratory temperature showed the absorption to be less in the latter series.   The same was true for all other salts.   It seems, therefore, that the *rate* of absorption for a specific solution and seed depends to a large extent on temperature.   This confirms the statement made by van Rijsselberghe, who studied the absorption power of young twigs and cells of Sambucus pith.

It is of interest to notice the stimulating effect of the different salts on the

germination of corn under constant temperature. The germination energy of the corn used was high. Germination was but slightly affected by distilled water. Absorption of $Ca(NO_3)_2$, NaCl and $MgSO_4$ solutions decreased with the increase of concentrations. The seeds soaked in $Ca(NO_3)_2$ produced the longest roots in the solutions of from 0.1 to 4 atmospheres, while even in the higher concentrations, with the exception of the solution of 7 atmospheres, larger roots were formed than in distilled water. This stimulating effect was very marked in the solution of 0.5 atmosphere, where the relative lengths of the roots were 131, and of the shoots 210 as compared with 100 in distilled water. A graphic representation of the results with $Ca(NO_3)_2$ is shown in figure 5.
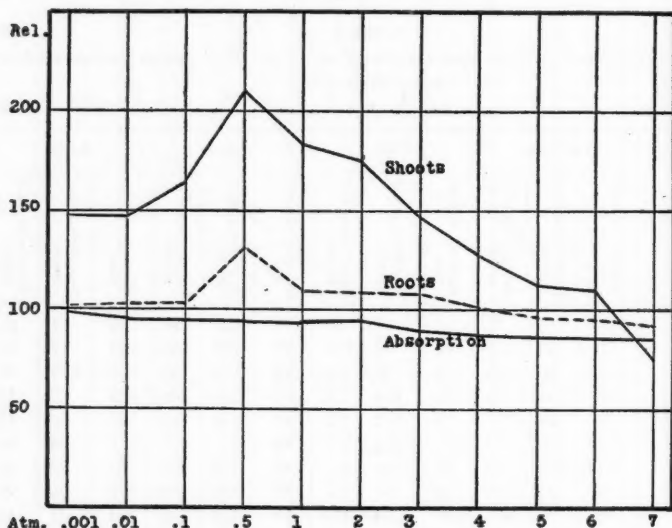
FIG. 5. RELATIVE ABSORPTION, ROOT- AND SHOOT-LENGTHS OF CORN SOAKED IN DIFFERENT OSMOTIC CONCENTRATIONS OF $Ca(NO_3)_2$
100 = distilled water

Sodium chloride also had a decidedly stimulating effect with concentrations of 0.5 atmosphere but reached its maximum at 2 atmospheres. The same effect was very evident upon the seeds soaked in $MgSO_4$. From the very dilute solutions up to the strongest concentrations, the seeds produced much longer roots and shoots than the seeds previously soaked in water. The vital activities were particularly stimulated in the solutions lower than 5 atmospheres. Corn seemed to be benefited when soaked in these salt solutions before planting. Potassium carbonate produced an entirely different type of effect. The attack upon the seed coats by this salt has been discussed elsewhere (16). In these experiments the germination and root-length diminished with the increase of

the osmotic pressure. The same was the case for the growth of the shoots. The absorption in the medium concentrations was greater than in the higher concentrations or in distilled water. In may be seen from the table that, in the series with these particular salt solutions, the roots are short as compared with the shoots. The average length, however, does not bring out the rather peculiar behavior of a number of the seedlings. Several produced shoots but no roots. In other instances only very short roots were produced, or the shoots appeared before the roots, and the root-growth was slight. The swollen seeds burst and a rather thick, obtuse, conical-shaped radicle of not more than 2- to 3-mm. length appeared. This rudimentary root remained at the same length while the shoots seemed to elongate normally. At the end of the period, some of the shoots were from 10 to 15 mm. long, and the roots 1 mm. Some of these seedlings were allowed to remain in the seedbeds, but while the shoots elongated, the roots made no effort to extend their length. The roots which made growth showed signs of injury, as a rule. The salt solutions seemed to have affected the seeds in such a way as to prevent root-growth. This phenomenon had been noticed in other salt solutions and for other seeds, but was accredited to the individuality of the particular seeds. Rape soaked in K_2CO_3 solutions behaved in a somewhat similar manner. The cotyledons appeared, grew slowly, but no root was put forth. When these seedlings were left in the seedbeds, the seed coats would drop in the same way as in ordinary germination. A number of these seedlings were placed in wet sand, but no difference occurred. After 6 to 10 days, the cotyledons showed signs of wrinkling, and succumbed slowly. When placed on wet sand in the light, the cotyledons made chlorophyll, and appeared normal, but finally reacted in the same way. No roots were formed.

In some instances, corn produced no main root. Instead, a bundle of from 3 to 5 secondary roots appeared together. This phenomenon was observed, though to a less extent, in seeds soaked in water, and still less frequently in unsoaked seeds. The salt seemed to stimulate this abnormal growth whereby roots were deformed to secondary roots.

Corn soaked in $Ca(NO_3)_2$, NaCl, and $MgSO_4$ behaved in an entirely different manner. The seeds soaked in $Ca(NO_3)_2$ produced a luxuriant root-growth. The main roots were strongly branched, and the root hairs appeared to be longer than normal. No separate measurements of the secondary roots were taken, but the stimulating effect of these salts on the vital activities is indicated in the tables.

In plant physiology it is a well-known fact that seedlings in solution without nitrogen make extremely long roots. This is ascribed to the so-called "nitrogen hunger" of the plants. It is doubtful, however, if this "hunger" could have so much influence on these young seedlings, especially in the case of $Ca(NO_3)_2$ solutions. The stimulating and retarding effects can be safely attributed to the influence of the soaking in the salts used.

The question can be raised as to whether the osmotic concentration inside

the seeds, as compared with the osmotic concentration of the salt, explains these phenomena, aside from the stimulating effect of the ions when they enter the seeds, and which become harmful when the absorption is too great. It has been pointed out before (16) that the seeds change the constitution of the salt solutions very quickly.

Influence of salt solutions upon the rapidity of germination

During the investigations reported above, a study was made to determine whether salt solutions stimulate germination, so that the radicles appear sooner. From the data obtained, only four series in solutions of two different salts are

TABLE 5

Influence of salt solutions upon the rapidity of germination

Seeds soaked for 15 hours

ATMOS-PHERES	NaNO₃							NaCl					
	Peas				Corn			Wheat			Alfalfa		
	Root length after:												
	24 hours	48 hours	72 hours	100 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
7	7	11	18	18	2	35	44	34	48	49	3	34	64
6	6	14	18	20	17	40	49	36	49	49	6	42	70
5	5	15	20	20	12	42	49	39	46	48	7	40	69
4	8	23	32	32	16	46	49	40	42	49	12	38	74
3	9	18	28	28	21	44	49	41	46	47	15	36	77
2	14	22	28	28	19	42	48	43	46	46	21	54	90
1	15	30	34	38	24	46	48	43	49	49	18	48	69
0.5	12	25	29	33	20	45	49	46	49	49	16	56	86
0.1	21	34	39	39	17	40	47	48	49	49	10	37	76
0.01	10	21	26	29	24	44	47	43	49	50	26	44	80
0.001	12	23	29	32	24	43	49	42	48	48	11	33	75
H₂O	20	25	28	31	23	45	49	37	49	49	11	52	82
Dry	0	2	28	40	0	3	45	0	48	48	0	45	81

given in table 5 as representative examples. Of the larger seeds, 50, and of the smaller seeds, 100, were soaked for 15 hours and germinated between filter papers. In the NaNO₃ series, absorption was less in the weaker solutions than in distilled water; in the NaCl series it was slightly greater. In the higher concentrations the absorption decreased gradually. At intervals of 24 hours the germinated seeds were counted and the roots measured. Root measurements only are given in the table, since in general the root-lengths corresponded to germination. The seeds were considered as germinated when the root tips broke through the seed coats. Bursting of seed coats might be considered as a more accurate beginning of germination, but this did not seem sufficient, as in many instances the seed coats were burst but no radicles appeared, and the seeds slowly rotted.

Peas soaked in NaNO$_3$ were retarded for the first 24 hours, as compared with those soaked in distilled water. This retarding effect was soon overcome, however, and during the second 24 hours, seeds soaked in some of the lower concentrations showed even better germination. During the third period of 24 hours, seeds in a number of the series germinated better and had somewhat longer roots than those in distilled water. After 100 hours no principal difference from the foregoing period occurred. None of the series showed a better germination power than the "dry" seeds which were germinated without previous soaking. In the higher concentrations a relatively small percentage survived. Apparently only the stronger seeds were able to withstand the influence of the salt solutions. Peas appeared to be more sensitive to salt solutions and soaking than did corn. Although germination of corn was retarded at first in the series of higher osmotic concentrations, it was soon equal to that in distilled water. The total germination after 72 hours was the same in most of the series.

The series in which the seeds were soaked in NaCl represent another type of absorption. For the first 24 hours, wheat germinated better in the series of from 0.001 to 5 atmospheres, while germination in the two highest concentrations was almost the same as that in distilled water. After 48 hours little difference occurred; the same was true after 72 hours.

In the alfalfa series germination was better after 24 hours in medium strong salt solutions, but this gain was lost after 48 hours. It appeared that peas, alfalfa, lupine, buckwheat, and watermelon are far more sensitive to salt solutions and to soaking than are corn and wheat. The use made of corn and wheat in agricultural research is far greater than that of other seeds; but when injurious effects are studied, more sensitive seeds seem better, as more pronounced results can be obtained.

SUMMARY

A study was made of the stimulating effects of salt solutions with known osmotic concentration upon seeds soaked in these solutions; of the injurious effects upon germination of seeds in continuous contact with salt solutions, as compared with germination in distilled water and of seeds which were not previously soaked; and the effect of temperature upon absorption, germination, and root- and shoot-growth of seeds kept in contact with the salt solutions. The salts used were: MgSO$_4$, NaNO$_3$, Ca(NO$_3$)$_2$, NaCl, K$_2$CO$_3$, KCl, KH$_2$PO$_4$, KNO$_3$, and Shive's three-salt solution. Concentrations ranged from 0.001 atmosphere to 7 atmospheres. To each series was added a control series in distilled water. The seeds used were: white lupine, watermelon, peas, buckwheat, soy beans, wheat, corn, beans, alfalfa, and rape. The seeds were germinated between double filter paper beds. The relative weight of the seeds, the relative absorption, and the relative length of roots and shoots are compared with each other and with the control series. The following is a summary of the observations made:

1. Previous soaking in distilled water was harmful to the germination of all seeds, corn being the least affected.

2. Stimulating effects:

a. With the relative decrease in absorption, germination and subsequently root-growth and top-growth decreased for all seeds.

b. A number of seeds absorbed more, thus making longer roots and shoots, in weak solutions than in dstilled water.

c. All seeds were greatly injured in K_2CO_3 solutions. In K_2CO_3 and $MgSO_4$ a number of different seeds produced no roots, but solely shoots; other abnormalities occurred.

d. $Ca(NO_3)_2$ had a detrimental effect upon the germination and root-length of nearly all seeds, except corn, which was stimulated in all concentrations as compared with seeds soaked in distilled water.

e. $MgSO_4$ stimulated corn, while lupine seeds were relatively less injured in $MgSO_4$ solutions than in other salt solutions.

f. Lupine germinated better and produced longer roots in medium solutions of $NaNO_3$.

g. It is possible that the osmotic concentration inside the seeds, as compared with the osmotic concentration outside in the salt solutions, causes greater absorption.

h. A comparison between the relative weight of the seeds and the relative length of the roots and shoots brings out the fact that the relative weight is of minor importance for early growth when seeds are soaked.

3. *a.* Absorption, germination, and root-growth decreased with the increase in concentration of the salt solutions as compared with distilled water, except for some of the weaker solutions.

b. K_2CO_3, in all concentrations employed, was injurious to roots.

c. With corn, KH_2PO_4 solutions favored absorption and produced relatively longer roots than shoots.

d. In general, constant contact with salt solutions is injurious to germination and root-growth.

4. Control of temperature and length of time made no principal difference in the relative absorption of salt solutions nor in root-growth, but affected the *rate* of absorption.

5. The influence of salt solutions upon the rapidity of germination is not the same for all seeds. Corn and peas soaked in $NaNO_3$ were retarded for the first 24 hours, but wheat and alfalfa germinated more quickly during the same period, as compared with germination in distilled water.

6. Peas, alfalfa, lupine, buckwheat and watermelon are far more sensitive to the influence of salt solutions than corn and wheat.

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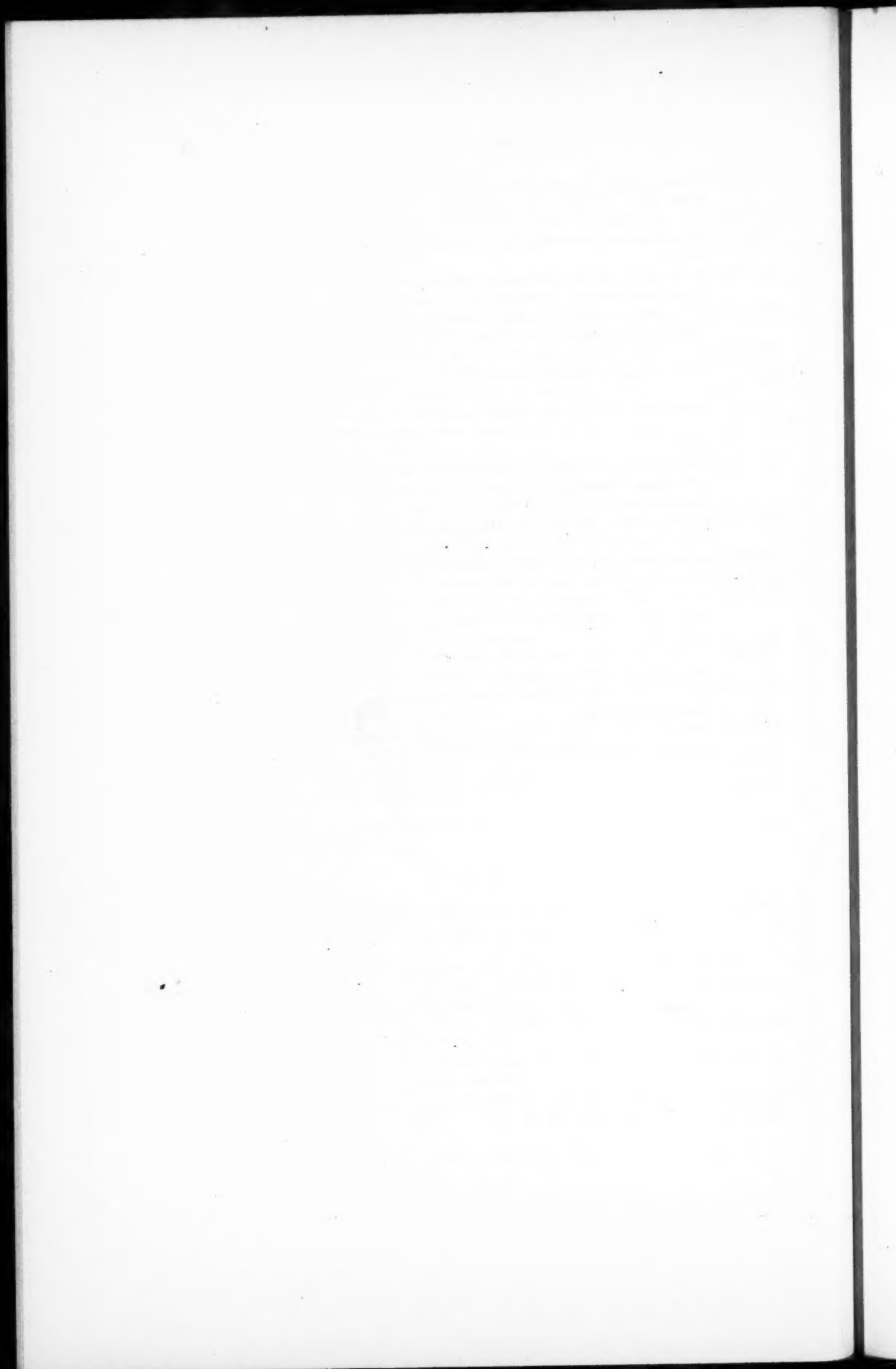
THE CARBON DIOXIDE CONTENT OF THE SOIL AIR AS A FACTOR IN THE ABSORPTION OF INORGANIC ELEMENTS BY PLANTS

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The question as to the importance of the carbon dioxide content of soil air as a factor in the absorption, by plants, of inorganic elements is not new; a number of studies already have been made bearing on the subject. The writer in a previous publication (1) reported the results of a series of experiments on the relation between the carbon dioxide production of plant roots and the feeding power of plants. In those experiments the carbon dioxide production of several crops was studied by three different methods. At the same time the feeding power of the plants was measured by determining the amount of Ca, Mg, K, P and N absorbed by the plants. A study of the two sets of data thus obtained showed rather conclusively that there was no relation between the amount of carbon dioxide given off by the plant roots and the amount of the inorganic elements absorbed by the plant. A study of the influence of removing the carbon dioxide from the soil on plant growth and the absorption of the inorganic elements indicated that the removal of carbon dioxide had practically no influence on growth or absorption by the plant.

In the experiments just mentioned, as none of the cultures received additions of carbon dioxide, the carbon dioxide content of the soil air was determined, almost wholly, by the amount given off by the plant roots. In continuation of the investigation a study has been made of the influence of adding carbon dioxide to the soil air.

In the previous experiments the cultures in every instance were unfertilized except for additions of sodium nitrate. Consequently absorption was almost wholly from the native soil material. It seemed possible that while variations in carbon dioxide production might not have much influence on the absorption of native soil material it might influence absorption of calcium or phosphorus from rock phosphate. Therefore, a study was made of the influence both of adding carbon dioxide to and of removing it from the soil air on the amount of calcium and phosphorus absorbed from cultures fertilized with rock phosphate.

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EXPERIMENT 1

The object of this experiment was to determine the influence of adding carbon dioxide to and of removing it from the soil air on the growth of the plant, and on the absorption of native soil material by the plant. Oats, rape, and crimson clover were grown in three series. In series 1 carbon dioxide was removed by rapid aspiration. Series 2 received no carbon dioxide treatment. In series 3 one gram of carbon dioxide was added to the soil twice a day. The detailed procedure was as follows:

All cultures were in 4-gallon earthenware jars, containing 6 plants per jar. The soil was similar to that used in the earlier experiments, a poor Norfolk sandy loam. All cultures received 1.0 gm. NH_4NO_3 at planting but no other fertilizer. The cultures were planted December 17, 1923, and were kept in an unheated greenhouse. Carbon dioxide was removed from series 1 by rapid aspiration. The method was the same as that used in the earlier experiments except that an electric pump was used and aspiration was more rapid. Carbon dioxide was added to the cultures of the third series, 1 gm. of CO_2 being introduced at the bottom of the culture twice each day. The CO_2 was obtained by the action of HCl on marble in a small CO_2 generator attached to each culture of the series. The amount of CO_2 evolved was regulated by the quantity of acid added. The plants were harvested May 2, 1924, and the dry weight, percentage of ash, calcium and phosphorus was determined. The results are shown in table 1.

The carbon dioxide treatment had very little influence on the dry weight of the plants. The highest yield of rape and crimson clover was obtained from the cultures receiving no carbon dioxide treatment. In the case of oats the highest yield was in the cultures to which carbon dioxide was added, this treatment causing an increased stooling. The differences in yield were not large but there was apparently a tendency for the yield to be reduced by the removal of carbon dioxide.

The differences in the ash content are not very significant except in the case of oats. In that instance the removal of carbon dioxide decreased the ash content and the addition of carbon dioxide produced a large increase. The character of the ash indicated that this difference was apparently due in large measure to variations in the silica content. A silica determination was made with the following very striking results: Plants from cultures where carbon dioxide was removed contained 0.87 per cent SiO_2, untreated plants 2.92 per cent, and where carbon dioxide was added, 5.62 per cent. In this experiment the high silica content was associated with vigorous stooling.

The carbon dioxide treatment had some influence on the calcium and phosphorus content of plants. In the case of rape and crimson clover the addition of carbon dioxide produced a small increase in the calcium and phosphorus content. The removal of carbon dioxide, on the other hand, caused a slight decrease in the phosphorus content. It is probable that phosphorus was the element limiting growth in these cultures.

The results of the experiment indicate that the carbon dioxide content of the soil is not an important factor influencing the absorption of inorganic

elements by plants.   The one exception to this was found in the absorption
of silica by oats.   That particular results should be verified by additional
experiments.

EXPERIMENT 2

The purpose of this experiment was to determine the effect of carbon dioxide
treatments on the absorption of calcium and phosphorus from rock phosphate.

TABLE 1

*Yield and percentage (dry weight) of ash, calcium and phosphorus in the plants grown with
the treatments indicated*

TREATMENT	RAPE	OATS	CLOVER
Dry weight of tops			
	gm.	gm.	gm.
CO$_2$ removed...............................	12.84	24.75	19.15
CO$_2$ not removed............................	13.83	28.79	25.87
CO$_2$ added...................................	10.90	30.35	20.75
Ash content			
	per cent	per cent	per cent
CO$_2$ removed...............................	11.09	5.31	13.28
CO$_2$ not removed..........................	10.65	7.32	17.42
CO$_2$ added...................................	11.37	10.78	14.98
Calcium content			
CO$_2$ removed...............................	2.54	0.37	1.48
CO$_2$ not removed...........................	2.40	0.39	1.42
CO$_2$ added...................................	3.03	0.34	1.71
Phosphorus content			
CO$_2$ removed...............................	0.095	0.095	0.095
CO$_2$ not removed...........................	0.109	0.099	0.097
CO$_2$ added...................................	0.118	0.097	0.113

For comparison the experiment included cultures receiving no phosphate
and cultures receiving acid phosphate.

Sorghum and cowpeas were grown in three series.   In series 1 carbon dioxide
was removed by aspiration as in the preceding experiment.   Series 2 did not
receive any carbon dioxide treatment.   In series 3 one gram of carbon dioxide
was added twice a day.   Each series contained two cultures without phosphate
treatment, two cultures receiving acid phosphate at the rate of 1000 pounds
per acre and two cultures receiving rock phosphate at the rate of 1000 pounds
per acre.   All cultures received 1.0 gm. of sodium nitrate at planting.   The
plants were grown in 2-gallon earthenware jars and the procedure was similar

to that followed in experiment 1. The plants were harvested September 11, 1924, after growing a period of 50 days.

The results obtained with cowpeas are given in table 2. With the three phosphate treatments the highest average yield was from the cultures having the carbon dioxide removed by aspiration. The differences were not large and are not considered significant.

The ash content of the cowpeas was not altered by carbon dioxide treatment except in the cultures receiving acid phosphate. Here as in the case of

TABLE 2

Yield and percentage (dry weight) of ash, calcium and phosphorus in cowpeas grown with the treatments indicated

TREATMENT	NO PHOSPHORUS	ACID PHOSPHATE	ROCK PHOSPHATE
Dry weight of tops			
	gm.	gm.	gm.
CO_2 removed..............................	13.68	16.18	14.84
CO_2 not removed...........................	10.40	15.42	12.91
CO_2 added...................................	11.40*	13.02*	12.62
Ash content			
	per cent	per cent	per cent
CO_2 removed..............................	8.30	10.90	9.90
CO_2 not removed...........................	9.30	12.00	10.30
CO_2 added...................................	8.80	13.20	10.30
Calcium content			
CO_2 removed..............................	1.37	2.20	1.75
CO_2 not removed...........................	1.46	2.25	1.84
CO_2 added...................................	1.53	2.82	1.97
Phosphorus content			
CO_2 removed..............................	0.105	0.272	0.208
CO_2 not removed...........................	0.108	0.280	0.226
CO_2 added...................................	0.102	0.225	0.227

* Dry weight is for one culture. The duplicate culture was injured by insects.

oats in the first experiment the removal of carbon dioxide caused a decrease and the addition of carbon dioxide, an increase in the ash content of the plant. However, the differences are not nearly so large as in the case of oats.

The results for calcium show small differences which are apparently due to the carbon dioxide treatment. With the three phosphate treatments the lowest calcium content was where carbon dioxide was removed and the highest calcium content was in the cultures receiving additions of carbon dioxide. The differences are not so large as those due to the phosphate treatment.

However, the regularity of the differences makes it seem probable that they can be attributed to the carbon dioxide treatment.

The differences in phosphorus content are small and do not follow a regular order as in the case of calcium. These results would lead to the conclusion that the absorption of phosphorus by cowpeas was not influenced by the carbon dioxide content of the soil air.

Table 3 gives the results obtained with sorghum. One striking difference between the results with sorghum and cowpeas was the response to the phos-

TABLE 3

Yield and percentage (dry weight) of ash, calcium and phosphorus in sorghum grown with the treatments indicated

TREATMENT	NO PHOSPHORUS	ACID PHOSPHATE	ROCK PHOSPHATE
Dry weight of tops			
	gm.	gm.	gm.
CO₂ removed.................................	22.56	42.35	44.16
CO₂ not removed.............................	29.41	41.28	40.61
CO₂ added...................................	18.18	40.71	45.88*
Ash content			
	per cent	per cent	per cent
CO₂ removed.................................	5.10	3.70	4.10
CO₂ not removed.............................	4.60	3.70	3.50
CO₂ added...................................	5.40	3.80	4.10
Calcium content			
CO₂ removed.................................	0.25	0.30	0.22
CO₂ not removed.............................	0.25	0.32	0.21
CO₂ added...................................	0.36	0.33	0.24
Phosphorus content			
CO₂ removed.................................	0.086	0.074	0.093
CO₂ not removed.............................	0.081	0.075	0.091
CO₂ added...................................	0.084	0.083	0.094

* Dry weight is for one culture. The duplicate culture was injured by insects.

phate treatment. The growth of sorghum was practically doubled by the addition of rock or acid phosphate but the growth of cowpeas was only slightly increased by the phosphate fertilization. With neither crop was the yield materially influenced by the carbon dioxide treatment.

The ash and phosphorus content of sorghum apparently was not influenced by the carbon dioxide treatment. The small differences obtained are so irregular that no importance can be attached to them. There may be some question as to whether or not the carbon dioxide treatment influences the

absorption of calcium. With the three sources of phosphorus the highest percentage of calcium was found where carbon dioxide was added. However, the differences were very small, except in the culture that did not receive phosphorus.

The results of the second experiment indicate that the availability of rock phosphate to a plant is not influenced by the carbon dioxide content of the soil air. From these results one might assume that the differences in the feeding power of plants for rock phosphate could not be attributed to differences in the carbon dioxide production of the plant roots.

SUMMARY

Two experiments are reported dealing with the influence of the removal from the soil air and the addition to it of carbon dioxide, on the absorption of inorganic elements by plants. In the first experiment, rape, oats and crimson clover were grown on a poor sandy soil without fertilizer additions other than nitrate. In the second experiment cowpeas and sorghum were grown in cultures receiving rock phosphate, acid phosphate and no phosphate. In all cases the yield, ash, calcium and phosphorus content were determined. The results obtained in the experiment may be summarized as follows:

1. The removal from the soil air or the addition to it of carbon dioxide did not materially influence the yield in either experiment.
2. The phosphorus content of the plant was slightly increased by the addition of carbon dioxide to the soil air under the conditions of the first experiment. The results of the second experiment indicate, however, that the availability of rock phosphate is not influenced by alterations in the carbon dioxide content of the soil air.
3. In most instances the carbon dioxide treatments did not influence the calcium content of the plant. However, in the case of cowpeas the addition of carbon dioxide apparently caused a slight increase in the calcium content.
4. In most cases the ash content of the plant was not influenced by the carbon dioxide treatment. The most striking exception was with oats where the addition of carbon dioxide increased and the removal of carbon dioxide reduced the percentage of ash.

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A STUDY OF VARIOUS STRAINS OF BACILLUS RADICICOLA FROM NODULES OF ALFALFA AND SWEET CLOVER[1]

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Early in the study of the nodule bacteria it was recognized that certain genera of Leguminosae required specific organisms for inoculation. The nodule bacteria were consequently separated on this basis into groups. The nodule bacteria possess few outstanding characteristics such as are commonly used in the differentiation of most bacteria. In many characters these bacteria show so much similarity that investigators have been led to believe that the various groups according to inoculation tests are only varieties of a single species.

Burrill and Hansen (3) proved that there were two distinct forms, morphologically and culturally, among the nodule bacteria. Hansen (5) in a later paper published the results of flagellation studies and pointed out that there were two arrangements of flagella; one, a single polar flagellum occurring on cowpea, soybean, hog peanut, and the other, peritrichous occurring on clover, sweet clover and vetch. Shunk (9) verified the work of Hansen as regards the flagellation of the nodule organisms, from most of the common legumes. Löhnis and Hansen (8) worked out a valuable method for determining the purity of cultures and for differentiating the two groups. The action of these organisms in milk was made the basis of separation.

Fred and Davenport (4) established differences in the critical pH of the nodule bacteria of the common legumes as shown below:

1. Critical pH 4.9—alfalfa and sweet clover
2. Critical pH 4.7—garden pea, field pea and vetch
3. Critical pH 4.2—red clover and common bean
4. Critical pH 3.3—soybean and velvet bean
5. Critical pH 3.15—lupine

Serological reactions which offered a new method of differentiation were first used by German investigators. In 1912, Zipfel (13) made use of the agglutination test in order to determine the relationship among the various nodule bacteria. From his results he concluded that distinct species existed

for the various groups of legume plants. Two years later Simon (10) made cross-inoculation tests and compared the results with those obtained by Zipfel. He found that the results of both methods agreed.

Klimmer and Krüger (7) employed the agglutination test, complement-fixation test and precipitin test in a study of 18 strains from 18 legume species. They separated the organisms into 9 distinct species which differed sharply from one another in their serological reactions. Vogel and Zipfel (12) in 1921 reported on similar studies. Their results agreed in general with those of Klimmer and Krüger, both investigations showing that the various natural groups could be separated by serological tests.

In 1923 the author reported the results of a serological study of 55 strains of nodule bacteria which represented 7 groups of the common legumes (11). The cultures used were from various sources and were tested for purity and ability to produce nodules. The cultures were separated into groups by means of the agglutination test, immune sera from rabbits being used. The 55 strains, which represented 7 cross-inoculation groups, were separated into 18 distinct serological groups which differed sharply from each other in their agglutination reactions. The cultures of pea-vetch nodule organisms studied were separated into 4 groups. The soybean cultures were also divided into 4 distinct groups, while the 13 cultures of alfalfa and sweet clover were separated into only 2 groups. Numerous tests indicated that the agglutination properties of the various cultures were fixed and constant.

These results are in agreement with those of Bialosuknia and Klott (1) who noted that the same kind of plant from different sources may have nodule bacteria with different agglutinating properties. They also report that different nodules on the same plant may contain serologically different bacteria. This last observation is of special interest and deserves careful study.

The results of the above investigations show that the nodule bacteria may be separated into groups by other means than the inoculation test. The mode of flagellation divides the organisms into two groups, the pseudomonas and the bacillus. These two groups can be recognized by cultivation of the organisms in milk. The bacillus group all form a serum zone while the pseudomonas group do not. The agglutination test, which is used widely in other fields of bacteriology for diagnostic work and for separating strains, indicated clearly that the nodule bacteria of even one kind of legume could be separated into several groups.

The results obtained by agglutination studies suggest the possibility of other methods for the separation of the various strains. Cultural studies conducted in connection with the previously reported serological studies indicated certain slight cultural differences among the various strains which seemed to be constant and to be correlated with serological differences. These slight cultural differences among the various serological groups were noted on various solid media and in litmus milk.

As a result of the above observations, more extensive laboratory and green-

house studies were planned to find means of grouping the various cultures of alfalfa and sweet clover bacteria.   The nitrogen-fixing power of the various strains has received special study.

EXPERIMENTAL

The organisms studied were from known cultures of bacteria isolated from the root nodules of alfalfa and sweet clover.   These cultures had been tested for purity and ability to cause nodule formation.   All of the strains were tested for the presence of *B. radiobacter* in milk and on potato, according to the method of Löhnis and Hansen (8).   Radiobacter gives a characteristic reaction in milk and on potato which is easily distinguished from that of the legume bacteria.   This method is also reliable in detecting other forms of contamination. Further tests for purity were made in plain broth and by microscopic examination.

A total of 25 strains were studied, but of these only 13 were used in the major part of the work.   Some of the strains had been cultivated in the laboratory away from the host plants for at least ten years.   Other strains had been away from the host plants only a year or two, while some were recently isolated.   These strains were secured from widely separated sources.

CULTURAL CHARACTERISTICS

The cultural characteristics of the 13 strains were studied on mannitol agar, yeast-water-mannitol agar, bean-extract agar, sucrose-nitrate agar, and on potato slants.   Two general types of growth were noted.   These characteristics were best shown on mannitol agar.   The most apparent differences were the rate and amount of growth.   Six strains produced an abundant, opaque, raised, pearly white growth with considerable slime; while 6 produced a thin growth with little slime as shown in table 1.   Strain 108 produced a growth somewhat intermediate between the two groups.   All of the strains failed to grow or showed very scanty growth on potato, except when the potato had been cooked for a very long time.

The fact that these organisms will not grow on properly prepared potato within a week or more, makes this medium valuable for diagnostic purposes. As many kinds of organisms will grow on potato, especially those most frequently found as contaminants in cultures of nodule bacteria, a culture of legume bacteria which shows growth on potato within a few days is usually contaminated.

Growth in peptone-sucrose solution of varying hydrogen-ion concentrations

A study was made with eight cultures in Buchanan's (2) peptone-sucrose solution of different reactions, ranging from pH 4.0 to pH 9.0.   The formula of the medium follows:

Mono-basic potassium phosphate (KH_2PO_4)......................   2.0 gm.
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$).............................   0.1 gm.
Peptone..   1.0 gm.
Sucrose..   20.0 gm.
Distilled Water.. 1000.0 cc.

The reaction of the medium was adjusted with sodium hydroxide or sul-
phuric acid. The sugar was sterilized in pure water and added to the other
ingredients. The reaction of the media was checked again after sterilization.
Ten-cubic centimeter portions were inoculated with a uniform amount of a
5-day-old culture.

The cultures were examined for growth after 1, 2, 3, and 4 weeks. The
turbidity of the medium, which naturally is very clear, was used as the measure
of the amount of growth. The growth in the tubes of pH 4.5 to 6.0 was
checked by transfers to mannitol agar slopes.

TABLE 1

Cultural characteristics of the nodule bacteria of alfalfa and sweet clover

After 2 weeks at 28°C.

STRAIN	SERO-LOGICAL GROUP	MANNITOL AGAR SLANT
Sweet clover 110, 111, 112	A	Abundant, opaque, raised, whitish growth. Considerable slime
Alfalfa 100, 107, 109..............	A	Abundant, opaque, raised, whitish growth. Considerable slime
Alfalfa 106......................	A	Moderate, opaque, thin, whitish growth
Alfalfa 101, 102, 103, 104, 105.....	B	Moderate, opaque, thin, whitish growth. Little slime
Alfalfa 108......................	B	Abundant, thin watery growth. Little slime. More translucent

At the end of 1 week the cultures of strains 100, 106, 107, and 111 (group A)
showed a fair amount of growth at pH 7.0 to pH 9.0; while strains 101, 102,
104, and 105 (group B) showed practically no growth in any of the tubes of
the same range. The relative growth of the various strains at the differ-
ent reactions at 4 weeks is shown in table 2. The strains of group A grew
equally well at pH 6.5 to pH 9.0, while those of group B grew best at pH 6.5.
The strains of group B showed growth at pH 5.5 after 4 weeks, while none of
the strains of group A had survived at this reaction. The strains of group B
produced slime strings on the sides of the tubes and throughout the medium,
while the other strains grew more evenly.

The limit of growth of strain 106 is shown in plate 1.

After the organisms had reached their maximum growth, their effect on
the reaction of the medium was determined. The titratable acidity was de-
termined by the use of 0.05 N NaOH, with phenolphthalein as the indicator.
The change in titratable acidity is shown in table 3.

In every case where there was appreciable growth, the titratable acidity was increased. The acid-resistant strains (group B) produced the greatest change at pH 7.0 and 7.5. The less acid-tolerant strains produced the greatest change at pH 7.0 to 8.0. In either case the greatest change was on the alkaline side or at neutrality.

TABLE 2

Growth of alfalfa and sweet clover bacteria in peptone-sucrose liquid medium of varying hydrogen-ion concentrations

After 4 weeks at 28°C.

STRAIN	SERO-LOGICAL GROUP	pH VALUES						
		5.0	5.5	6.0	6.5	7.0	8.0	9.0
Alfalfa 100..........	A	−	−	+	++++	++++	++++	++++
Alfalfa 106..........	A	−	−	++	++++	++++	++++	++++
Alfalfa 107..........	A	−	−	++	++++	++++	++++	+++
Sweet clover 111.....	A	−	−	++	++++	+++	+++	+++
Alfalfa 101..........	B	−	+−	+	+++	++	++	++
Alfalfa 102..........	B	−	+−	++	+++	++	++	++
Alfalfa 104..........	B	−	+−	++	+++	++	++	++
Alfalfa 105..........	B	−	+−	+	+++	++	++	++

−, no growth; +, fair; ++, medium; +++, good; ++++, excellent.

TABLE 3

Increases in titratable acidity due to the growth of alfalfa and sweet clover bacteria at varying initial reactions

After 7 weeks at 28°C.

STRAIN	SERO-LOGICAL GROUP	INITIAL pH VALUES					
		5.5	6.0	6.5	7.0	7.5	8.0
		Increase in acidity 0.1 N acid in 100 cc. of culture					
		cc.	cc.	cc.	cc.	cc.	cc.
Alfalfa 100	A	0.0	1.5	1.50	2.75	3.50	4.75
Alfalfa 106	A	0.0	1.0	1.25	3.75	4.00	4.25
Alfalfa 107	A	0.0	1.0	2.50	5.75	5.25	6.75
Sweet clover 111...........	A	0.0	1.0	2.00	5.75	4.50	5.25
Alfalfa 101	B	0.0	1.0	2.00	3.75	2.50	1.75
Alfalfa 102	B	0.0	0.0	1.00	2.75	4.00	2.75
Alfalfa 104	B	0.0	1.5	2.00	3.75	3.50	2.25
Alfalfa 105	B	0.0	0.5	1.50	1.75	3.50	2.75

The change in hydrogen-ion concentration was in accord with the change in titratable acidity. The greatest increase in hydrogen-ion concentration was 1.1 and was in a medium of an initial reaction of pH 9.0. All of the strains produced the greatest change at pH 7.0 or above. Strains of group A caused the greatest change in hydrogen-ion concentration.

On the basis of behavior toward acidity, the 8 strains may be separated into two groups which differ in their ability to change the titratable acidity and hydrogen-ion concentration of the medium at varying pH values.

<div align="center">SEROLOGICAL STUDIES</div>

Detailed serological studies were made of 13 cultures. An agglutinating serum was obtained by injecting rabbits intraperitoneally with a physiologic salt suspension of the desired organism washed from a 4-day agar slant. To prepare the suspensions, 10 cc. of 0.85 per cent salt solution was used with each culture. Three injections of 1, 2 and 3 cc. respectively, were given at 3-day intervals. Occasionally four or five injections were necessary with some animals.

One week after the last injection, samples of blood were drawn directly from the heart by means of sterilized needles. The blood was placed in sterilized test tubes and allowed to stand in the ice box 24 hours. If the serum had not separated, it was centrifuged. Suspensions of bacteria for use in testing the serum were prepared with physiologic salt solution, filtered, and standardized before they were used. Phenol, 0.5 per cent was added as preservative.

The agglutinating power of the serum was tested as follows:

To a series of small test tubes (⅜ by 3 inches) 1-cc. portions of a suspension of the desired organism were added. Serum was added to the 1-cc. portions of antigen so that dilutions of 1:50, 1:250, 1:500, 1:1000, 1:2000, 1:5000, 1:7500, 1:10,000, 1:20,000, 1:30,000 and 1:40,000 were obtained. Dilutions below 1:50 were found of little value and were discarded. The serum and antigen were well mixed and incubated at 37°C. As controls 1-cc. portions of the antigen without the serum were used. The results were determined after 24 hours, as the reaction was usually complete before this time.

Little difficulty was encountered with this procedure in obtaining sera of high titer. Complete agglutination in dilutions of 1:20,000 to 1:30,000 was noted with cultures of these organisms. Old laboratory cultures seemed to produce higher titers than freshly isolated ones. Some rabbits were used which would not produce agglutinins in appreciable quantities even after repeated injections. Too much antigen may have been injected.

The 13 cultures used were separated into two distinct groups by cross-agglutination tests as shown in table 4. The two groups showed little relationship in their serologic reactions. Some cultures of group A (100, 106, 107, 109, 110, 111 and 112) would react in low dilutions with cultures of group B (101, 102, 103, 104, 105 and 108), as shown in table 6. Sera from rabbits immunized with these organisms would not react with cultures of nodule bacteria isolated from other legumes, as clover, except occasionally in low dilution (tables 4 and 5). Cultivation on different media and growth in soil for 9 months did not change the serological characteristics of the cultures.

Sera used in the above experiments were later tested against other cultures of nodule bacteria isolated from alfalfa and sweet clover nodules, but no reac-

tion was noted.   Some of these were old cultures obtained from other laboratories.   This observation indicates that more groups may be found if a greater number of cultures are tested.

From all of these results it is evident that the nodule bacteria of alfalfa and sweet clover can be separated by means of the agglutination test into two or more groups.   The serologic differences between these groups are almost as

TABLE 4

Grouping of the alfalfa and sweet clover bacteria by means of serum of rabbit immunized with sweet clover strain 111

ANTIGENS	AGGLUTININ TITERS
Sweet clover 111, 110..	20,000
Sweet clover 112..	1,000
Alfalfa 100, 106, 107...	2,000
Alfalfa 109...	1,000
Alfalfa 101, 102, 103, 104, 105, 108..........................	0
Red clover 121, 122, 123, 124, 125............................	0
Garden pea 131, 132, 133.....................................	0
Garden bean 140..	0
Lima bean 142..	0
Soybean 163, 164, 165, 166...................................	0

TABLE 5

Grouping of the alfalfa and sweet clover bacteria by means of serum of rabbit immunized with alfalfa strain 102

ANTIGENS	AGGLUTININ TITERS
Alfalfa 102, 103, 104...	30,000
Alfalfa 101, 105..	20,000
Alfalfa 108...	10,000
Alfalfa 100, 106, 107, 109....................................	0
Sweet clover 110..	50
Sweet clover 111, 112...	0
Red Clover 121, 122, 123, 124, 125, 126.......................	0
Garden pea 130, 132, 133, 135.................................	0
Vetch 134..	0
Lima bean 142..	0
Cowpea 143	0
Garden bean 140..	0
Soybean 161, 163, 165..	0

sharp as the differences between groups as determined by cross-inoculation tests.   Because of these specific serological properties of the different cultures, the agglutination test is of value chiefly as a means of separating strains of a single natural group.   These differences in cultures, although not associated with the nodule-forming behavior of the organism, indicate differences in the inherent nature of the organism which may be of considerable value.

TABLE 6

Grouping of the alfalfa and sweet clover bacteria by means of immune sera

ANTIGENS	ANTISERA AND TITERS												
	100	106	107	109	110	111	112	101	102	103	104	105	108
Alfalfa 100........	1:20,000	1:5,000	1:10,000	1:5,000	1:500	1:2,000	1:1,000	0	0	0	1:100	0	0
Alfalfa 106........	1:10,000	1:10,000	1:2,000	1:2,000	1:500	1:2,000	1:500	0	0	0	1:100	0	0
Alfalfa 107........	1:10,000	1:5,000	1:10,000	1:7,500	1:500	1:2,000	1:2,000	0	0	0	1:100	0	0
Alfalfa 109........	1:20,000	1:5,000	1:2,000	1:2,000	1:500	1:1,000	1:2,000	0	0	0	0	0	0
Sweet clover 110...	1:5,000	1:5,000	1:2,000	1:7,500	1:7,500	1:20,000	1:2,000	1:250	1:50	1:250	1:50	1:50	0
Sweet clover 111...	1:10,000	1:5,000	1:2,000	1:2,000	1:7,500	1:20,000	1:2,000	1:250	0	1:250	1:250	0	0
Sweet clover 112...	1:1,000	1:2,000	1:2,000	1:250	1:250	1:1,000	1:2,000	0	0	1:100	1:50	0	0
Alfalfa 101........	1:100	0	0	0	0	0	0	1:10,000	1:20,000	1:10,000	1:5,000	1:2,000	1:250
Alfalfa 102........	0	1:50	0	0	0	0	0	1:10,000	1:30,000	1:20,000	1:10,000	1:2,000	1:500
Alfalfa 103........	0	1:100	0	0	0	0	0	1:7,500	1:30,000	1:30,000	1:10,000	1:2,000	1:500
Alfalfa 104........	1:100	1:100	0	0	0	0	0	1:10,000	1:30,000	1:30,000	1:10,000	1:2,000	1:500
Alfalfa 105........	0	1:100	0	0	0	0	0	1:10,000	1:20,000	1:20,000	1:10,000	1:2,000	1:500
Alfalfa 108........	0	1:100	0	0	0	0	1:250	1:2,000	1:10,000	1:2,000	1:2,000	1:500	1:2,000

NITROGEN FIXATION WITH VARIOUS STRAINS IN SAND CULTURES

The results of the previous experiments show that the nodule bacteria of alfalfa and sweet clover differ in certain characteristics, and that these differences arrange the organisms into groups which have no relation to the nodule-forming behavior of the organisms. A comparison of the groups as determined by the different methods shows that they are practically identical in each case. Such consistent differences between the two groups indicate that the organisms are fixed in their characteristics and probably represent two biological types of the same organism. The close correlation between the characteristics of the groups as brought out by the different methods of study, suggested the probability of corresponding differences in ability to fix nitrogen. The experiments which follow were an attempt to determine the relation between the nitrogen-fixing power and the other characteristics of the two groups.

To simplify the work, only a few cultures were selected from each group for this study. Four representative strains (100, 106, 107, and 111) from group A, and four strains (101, 102, 104, and 105) from group B were used. The four strains of group B were practically identical in their behavior under various conditions, while the four strains of group A showed considerably less uniformity in their various reactions.

Experiment 1—1922

The effect of the various cultures on alfalfa was studied in sand cultures. Ten-kilo portions of the well-mixed sand were weighed out into 2-gallon jars. To each jar 20 gm. of precipitated calcium carbonate was added and mixed thoroughly with the sand. The jars were then moistened with distilled water, covered with paper and sterilized for 4 hours at 20 pounds pressure.

Eighteen jars were planted with 50 seeds per jar of bacterial free Grimms alfalfa seed. Cultures of the desired strain of *B. radicicola* were sprinkled over the sand after the seeds were planted. The plan follows:

Number of jar

Uninoculated.. 3
Sweet clover 111... 3
Alfalfa 100.. 3
Alfalfa 106.. 3
Alfalfa 104.. 3
Alfalfa 102.. 3

When 2 or 3 inches high, the plants were thinned to 10 in each jar.

Hopkins and Pettit's (13) nutrient solution as given below was added to the jars in equal portions at different times throughout the growing period.

1. Monocalcium phosphate $(CaH_4(PO_4)_2)$......................... 10.0 gm.
   Distilled water... 1000.0 cc.
2. Potassium sulfate (K_2SO_4)................................... 20.0 gm.
   Distilled water... 1000.0 cc.

3. Magnesium sulfate ($MgSO_4 + 7H_2O$)..........................   8.0 gm.
      Distilled water.. 1000.0 cc.
4. Ferric chloride ($FeCl_4 + 6H_2O$)...............................   0.1 gm.
      Distilled water...   250.0 cc.

A total of 20 cc. each of solutions 1, 2, and 3, and 2 cc. of 4 were added to each jar during the growing period. The moisture content of the different jars was kept as near constant as possible with sterilized distilled water.

During the early growth of the plants no difference except slight variations in the height of the plants was noted between the different jars. All of the plants grew normally, appearing green and vigorous. However, by the six-tieth day, a difference in color was evident between the plants inoculated with cultures of the two groups. Plants inoculated with cultures of group B were lighter in color than those inoculated with organisms of group A. The size of the plants was nearly equal. The plants were cut at this stage.

The plants of the second crop were allowed to attain a greater height before they were cut. When 100 days old, at the date of the second cutting, it was very evident that the plants inoculated with the group A organisms were re-ceiving more benefit from the inoculation than those inoculated with the group B organisms. The plants were larger and darker in color. The height, color, general appearance and green weight of the plants of each jar were determined. The roots were washed and examined for nodules and the green weight was determined.

The samples collected in this manner were dried and weighed on an analyt-ical balance. For the first cutting, the triplicate samples were mixed together and analyzed as one sample, while for the second cutting the plants of each jar were analyzed separately. The plants were ground in a mechanical mortar to pass a 100-mesh sieve. For total nitrogen determinations the material was mixed well and dried over night at 105°C. For moisture determinations $2\frac{1}{2}$-gm. portions were dried. Duplicate 0.5-gm. portions of tops and 0.8-gm. portions of roots were used for total nitrogen determinations by the Kjeldahl method. The results are shown in tables 7 and 8.

The results obtained in this experiment are striking. A careful examina-tion of the roots of the plants showed an abundance of nodules in every case, with the exception of the uninoculated jars which were free from nodules with the exception of one jar which showed one or two large nodules. The roots of the plants inoculated with the cultures of group B showed more nodules than those inoculated with group A.

The superiority of type A inoculation manifested itself early in the growth of the plants. Although the water-free weights of the first cutting were practically the same, the nitrogen content was different. As seen in table 7 plants inoculated with strains 111, 100 and 106 (group A) were considerably higher in percentage of nitrogen than those inoculated with strains 102 and 104 (group B). The differences in yield, nitrogen con-tent and general appearance were more marked for the second cutting.

The plants inoculated with group A organisms were dark green and, when cut, were 17 inches high, while the plants inoculated with group B organisms were lighter green and averaged only 13 inches high. These differences in yield and nitrogen content are shown in table 8. The yield and nitrogen content of the roots also are shown. If it is assumed that the nitrogen benefit derived from the seed and sand is the same in each case, then the total nitrogen fixed in the one case is almost twice as much as in the other.

TABLE 7

Effect of various strains of alfalfa and sweet clover bacteria on the yield and nitrogen content of alfalfa

First cutting, 1922; 10 plants in each jar

NUMBER	STRAIN	SERO-LOGICAL GROUP	TOTAL WEIGHT 30 PLANTS DRY	TOTAL NITROGEN 30 PLANTS	NITROGEN
			gm.	*mgm.*	*per cent*
1	Sweet clover 111...............	A	4.442	149.7	3.37
2	Alfalfa 100....................	A	4.904	156.4	3.19
3	Alfalfa 106....................	A	5.164	176.6	3.42
4	Alfalfa 104....................	B	4.645	146.5	3.15
5	Alfalfa 102....................	B	4.345	133.8	3.08

TABLE 8

Effect of various strains of alfalfa and sweet clover bacteria on the yield and nitrogen content of alfalfa

Second cutting, 1922; 10 plants in each jar

NUMBER	STRAIN	SERO-LOGICAL GROUP	TOTAL WEIGHT 30 PLANTS DRY		TOTAL NITROGEN 30 PLANTS	NITROGEN	
			Roots	Tops		Roots	Tops
			gm.	*gm.*	*mgm.*	*per cent*	*per cent*
1	Sweet clover 111......	A	12.198	10.392	458.7	1.52	2.63
2	Alfalfa 100...........	A	14.395	11.382	512.2	1.55	2.54
3	Alfalfa 106...........	A	13.294	10.488	472.1	1.54	2.55
4	Alfalfa 104...........	B	7.902	8.250	291.3	1.40	2.19
5	Alfalfa 102	B	7.059	7.333	250.1	1.33	2.13

From the data of this experiment it is evident that the acid-sensitive organisms of serologic group A are more beneficial to the plants than are the acid-resistant strains of serologic group B. The differences are consistent in every case and indicate that the nitrogen-fixing behavior of the different strains is closely related to their reaction toward acid and to their agglutination and other specific properties.

Experiment 2—1923

The procedure for this experiment was essentially the same as for experiment 1. The jars were inoculated as follows:

In this experiment only 10 plants were left in each jar. An equivalent of 14.5 cc. of nutrient plant solutions 1, 2 and 3, and 1.4 cc. of 4 were added in the three applications. The other treatments were the same as for experiment 1.

An early difference was noted between the plants inoculated with the two groups of cultures. To show this difference, representative jars from each set of three were selected to be photographed at the end of 124 days. Plate 2, figure 1, shows the differences in growth of the first crop. After about 140 days the plants became so badly infested with red spider that the experiment could not be continued.

The plants were harvested 149 days from the date of planting. The height, color and green weight of the plants were determined. The green weight and the number of nodules were noted in each case. The tops were harvested separately at this time and the green weights recorded.

The tops of the first cutting from each jar were analyzed separately. The nitrogen results shown in table 9 are the average figures of the analysis of the three jars of 30 plants. The roots of the triplicate jars were analyzed as one sample.

A difference was noted earlier than in the previous experiment in the size, color and general vigor of the plants inoculated with the two groups of organisms. The plants inoculated with strains 100, 106, 107 and 111 (group A) were darker and taller. This difference became more noticeable as the plants developed. When the first harvest was made, the plants of group A were several inches taller than those of group B and were considerably darker. While the damages by the insects no doubt had considerable effect on the nitrogen content of the plants, the differences in yield and total nitrogen were great. As seen in table 9 the total yield and nitrogen were much greater in case of the plants inoculated with strains 100, 106, 107 and 111. While the percentage of nitrogen was about the same in the case of the jars inoculated with strains 104 and 102 as with jars inoculated with group A strains, the plants inoculated with strains 101 and 105 were considerably lower in nitrogen content (table 9).

The differences in yield and total nitrogen in the roots corresponded to the differences in the tops of the first cutting. The nitrogen content, however, averaged 2.06 per cent for roots of group B as compared with 1.74 per cent for roots of group A. These data are shown in table 9.

An examination of the roots showed that all of the plants were well inoculated. However, there was a difference in the number, size and distribution of the nodules. Plants inoculated with strains of group A showed many large nodules which were located chiefly within the first few inches of sand. The plants inoculated with group B strains bore many more nodules, which were very small and located in all parts of the root system. The uninoculated plants were free from nodules.

This tendency of group B strains to form numerous small nodules on all parts of the root system has been noted in connection with other inoculation tests in sand cultures in the greenhouse.

This experiment again shows the superior power of strains of group A to benefit the host plant. This superiority was not due to lack of nodule formation by strains of group B. Numerous greenhouse tests have shown that strains

TABLE 9

Effect of various strains of alfalfa and sweet clover bacteria on the yield and nitrogen content of alfalfa

First cutting, 1923; 10 plants in each jar

NUMBER	STRAIN	SERO-LOGICAL GROUP	TOTAL WEIGHT 30 PLANTS DRY		TOTAL NITROGEN 30 PLANTS	NITROGEN	
			Roots	Tops		Roots	Tops
			gm.	*gm.*	*mgm.*	*per cent*	*per cent*
1	Sweet clover 111......	A	6.719	10.621	387.9	1.68	2.59
2	Alfalfa 100......... ..	A	13.165	12.942	578.6	1.79	2.65
3	Alfalfa 106...........	A	9.704	11.070	454.1	1.66	2.63
4	Alfalfa 107...........	A	6.926	9.813	382.5	1.84	2.60
5	Alfalfa 104...........	B	2.942	5.712	212.5	2.16	2.61
6	Alfalfa 102	B	2.813	6.078	220.4	2.22	2.60
7	Alfalfa 101...........	B	3.931	6.596	230.5	1.84	2.41
8	Alfalfa 105...........	B	3.685	5.181	180.2	2.01	2.24

of group B cause nodules to form just as early as do strains of group A. In this experiment group B strains actually caused more nodule formation than did strains of group A.

Experiment 3—1924

The main plan of this experiment was the same as in the previous one. Certain factors, however, were given more consideration. It was realized that differences which were evident in the early growth of the plants might disappear in the later periods of growth. A second factor considered was the natural variations found in individual plants of alfalfa. An attempt was made to eliminate these two factors by extending the experiment over a longer period of time, and by leaving 20 plants in each jar, or a total of 60 plants in the triplicate jars.

The plants were cut at different periods to determine the effect of the various strains of nodule bacteria on the yield and nitrogen content of the plants at the various stages of growth.   The first cutting was made after 71 days, the second after 151 days, and the third after 212 days of growth.   The roots were removed from the jars at the time of the third cutting.

Except for the first, the entire cutting from each jar was ground and treated as a single sample.   This means that a complete analysis was made of the plants from each jar for the second and third harvests and for the roots.   The amount of material obtained from each jar in the first cutting was so small that the plants from the three similar jars were analyzed as one sample.   Numerous samples were chosen at random from the crops of 1922, 1923, and from this year's harvests and analyzed again as a final check against the original analysis.

TABLE 10

Effect of various strains of alfalfa and sweet clover bacteria on the yield and nitrogen content of alfalfa

First cutting, 1924; 20 plants in each jar

NUMBER	STRAIN	SERO-LOGICAL GROUP	TOTAL WEIGHT 60 PLANTS DRY	TOTAL NITROGEN 60 PLANTS	NITROGEN
			gm.	*mgm.*	*per cent*
1	Sweet clover 111....................	A	2.080	69.2	3.33
2	Alfalfa 100........................	A	1.388	45.7	3.29
3	Alfalfa 106........................	A	1.837	59.0	3.21
4	Alfalfa 107........................	A	2.110	66.5	3.19
5	Alfalfa 104	B	1.342	41.9	3.12
6	Alfalfa 102........................	B	1.647	48.1	2.86
7	Alfalfa 101........................	B	1.215	37.7	3.01
8	Alfalfa 105........................	B	2.047	61.8	3.02

The results of this experiment confirmed those previously obtained.   The first cutting showed differences in the percentage of nitrogen although the yields were nearly the same (table 10).   The average nitrogen content of the plants inoculated with group A organisms was 3.33 per cent as compared to 3.00 per cent for those inoculated with group B organisms.   The second harvest showed differences in both yield and percentage of nitrogen.   The plants inoculated with group A strains averaged 17 inches in height and were darker than those inoculated with group B strains.   The plants of the latter group averaged only 15 inches in height.   This difference in size and color is shown in plate 2, figure 2.   The yield, percentage of nitrogen, and total nitrogen are given in table 11 for the plants inoculated with the different strains.   As seen in this table, the plants of group A were consistently higher in yield and percentage of nitrogen.   The average yield was 22.52 gm. for group A as compared with 17.57 gm. for group B.   The average nitrogen content was 2.96 per cent for group A but only 2.80 per cent for group B.

The differences in effect of the two groups of organisms on the plants were still evident at the time of the last cutting, although the jars had become so root-bound that the plants could scarcely continue growing. As seen in table 12, the yield, percentage of nitrogen, and total nitrogen were greater for group A than for group B. The yield and nitrogen of the roots are also shown

TABLE 11

Effect of various strains of alfalfa and sweet clover bacteria on the yield and nitrogen content of alfalfa

Second cutting, 1924; 20 plants in each jar

NUMBER	STRAIN	SERO-LOGICAL GROUP	TOTAL WEIGHT 60 PLANTS DRY	TOTAL NITROGEN 60 PLANTS	NITROGEN
			gm.	*mgm.*	*per cent*
1	Sweet clover 111....................	A	21.617	665.8	3.08
2	Alfalfa 100.........................	A	23.499	683.8	2.91
3	Alfalfa 106.........................	A	22.985	675.8	2.94
4	Alfalfa 107.........................	A	21.997	651.1	2.96
5	Alfalfa 104.........................	B	18.000	509.4	2.83
6	Alfalfa 102.........................	B	17.491	478.0	2.74
7	Alfalfa 101.........................	B	15.881	451.0	2.84
8	Alfalfa 105.........................	B	18.927	530.0	2.80

TABLE 12

Effect of various strains of alfalfa and sweet clover bacteria on the yield and nitrogen content of alfalfa

Third cutting, 1924; 20 plants in each jar

NUMBER	STRAIN	SERO-LOGICAL GROUP	TOTAL WEIGHT 60 PLANTS DRY		TOTAL NITROGEN 60 PLANTS	NITROGEN	
			Roots	Tops		Roots	Tops
			gm.	*gm.*	*mgm.*	*per cent*	*per cent*
1	Sweet clover 111......	A	46.960	18.989	1,681.3	2.21	3.39
2	Alfalfa 100...........	A	53.480	18.855	1,734.6	2.05	3.41
3	Alfalfa 106...........	A	59.645	20.103	1,934.4	2.07	3.49
4	Alfalfa 107...........	A	52.354	20.243	1,828.0	2.17	3.42
5	Alfalfa 104...........	B	38.924	15.781	1,324.4	2.09	3.24
6	Alfalfa 102...........	B	49.365	17.697	1,547.6	1.96	3.30
7	Alfalfa 101...........	B	40.675	15.873	1,399.0	2.14	3.32
8	Alfalfa 105...........	B	49.573	16.990	1,573.5	2.01	3.40

in table 14. There was very little difference in the percentage of nitrogen in the different roots.

The results of this experiment showed without exception that organisms of group A were more beneficial to the plant than were organisms of group B. The superiority of group A strains to group B strains persisted throughout the experiment.

From the results of these three experiments, it was concluded that the nitrogen-fixing ability of strains of nodule bacteria of the alfalfa sweet clover group differ, and that this property is apparently constant for a given strain. On the basis of their ability to fix free nitrogen and benefit the host plant, the eight strains were arranged into two groups. The differences in ability to fix nitrogen showed a direct relation to other specific properties. The strains which were similar in their behavior toward hydrogen-ion concentration and in their serologic properties were also similar in their ability to fix nitrogen.

DISCUSSION

The possibility of other methods of measuring the ability of specific strains of nodule bacteria to benefit the host is not improbable. The desirability of a simple and quick method is very evident, but it is realized that the present knowledge of the nodule bacteria of any particular group is far from sufficient to warrant the application of any single method for the selection of high nitrogen-fixing strains. Extensive studies by different methods of many strains of the different cross-inoculation groups and comparisons of the results obtained with nitrogen-fixing studies, may lead to a simple and rapid method of determining the nitrogen-fixing ability of different cultures.

The primary purpose of the experiments reported in this paper was to determine if differences do exist among cultures of a single species in their ability to fix nitrogen and in other characteristics.

Serological studies show clearly that organisms of a single natural group can be separated into two or more groups which have little relation to each other. The 13 strains isolated from nodules of alfalfa and sweet clover were separated by cross-agglutination tests into two distinct groups. Sera from rabbits immunized with strains of either group would not agglutinate cultures isolated from red clover, soybean and other different legumes.

A comparison of the cultural characteristics of the 13 strains on different kinds of solid and liquid media showed certain constant variations in their growth. The most apparent difference was noted in amount and rate of growth. The 13 cultures fall into two general groups; the strains of one produced abundant, raised, opaque pearly-white growth, while strains of the second produced a moderate, thin, whitish growth. In general these differences were in accord with the serological differences. No changes in the characters of these organisms were noted over a period of 3 years of study.

On the basis of their behavior towards acidity 8 strains, representing the two serological groups, were separated into two groups. The two groups differed in their ability to survive under acid conditions and to change the titratable acidity and hydrogen-ion concentration of the medium. The acid-resistant strains grew best at pH 6.5 and caused less increase in titratable acidity and hydrogen-ion concentration than did the acid-sensitive strains which grew equally well at pH 6.5 to 9.0. The acid-resistant strains were

"slow growers" and were of serological group B, while the acid-sensitive strains were "fast growers" and belonged to serological group A.

Studies of the nitrogen-fixing powers of 8 strains in sand cultures in the greenhouse showed, without exception, that the plants inoculated with strains of serological group A were benefited more by the inoculation than were those inoculated with strains of serological group B. The superiority of strains of group A was evident early in the development of the plants and persisted throughout the experiments. In general the group A strains fixed almost twice as much nitrogen as did the group B strains.

The results of these experiments show that the nodule bacteria of alfalfa and sweet clover differ in their behavior under various conditions and that these differences are manifestations of inherent differences in the nature of the organisms.

CONCLUSIONS

1. Strains of the alfalfa sweet clover group of nodule bacteria differ under similar conditions in their ability to fix free nitrogen and to benefit the host plant.

2. Differences in the cultural characteristics of the nodule bacteria from the alfalfa sweet clover group have been demonstrated. The physiological, cultural and serological characteristics for a given strain do not change in spite of changes in environment and cultivation over a long period of time on different media.

3. The agglutination test separates these organisms into two or more groups which show little or no serological relation to each other.

4. Organisms of the alfalfa sweet clover group are not related serologically to nodule bacteria of the other cross-inoculating groups.

5. The various strains of alfalfa and sweet clover bacteria differ in their resistance to acidity.

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PLATE 1

SHOWING THE LIMIT OF GROWTH OF ALFALFA STRAIN 106 IN SUCROSE-PEPTONE SOLUTION
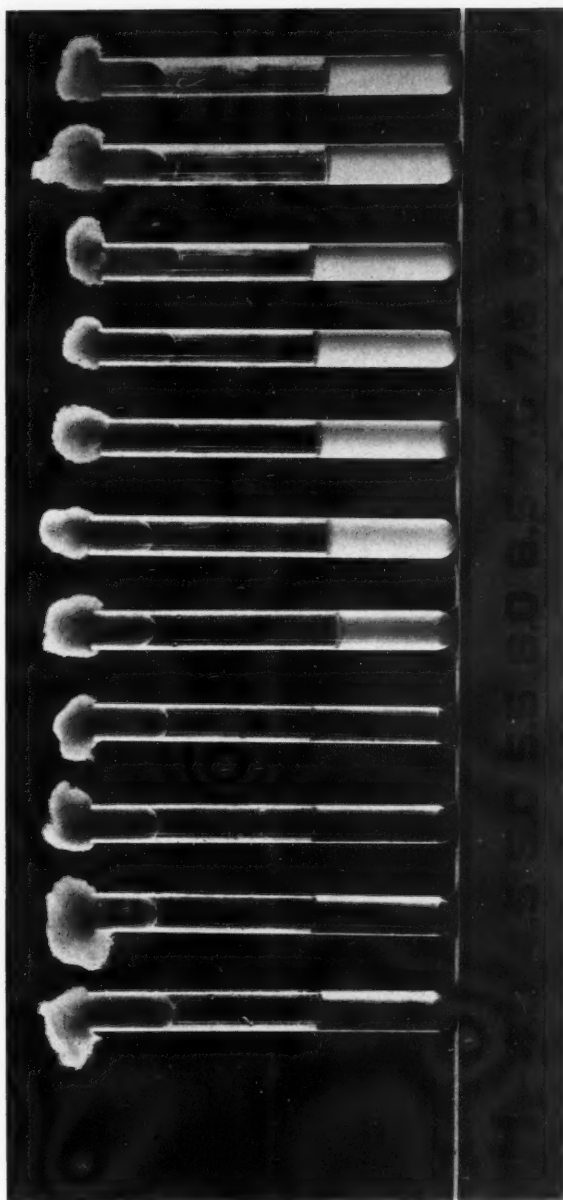
PLATE 2

FIG. 1. Showing differences in growth of first crop of alfalfa, 1923, inoculated with strains of serological groups A and B.   Group A is at the right.

FIG. 2. Showing the differences in growth of the second crop of alfalfa, 1924, inoculated with strains of serological groups A and B.   Group A is at the right.
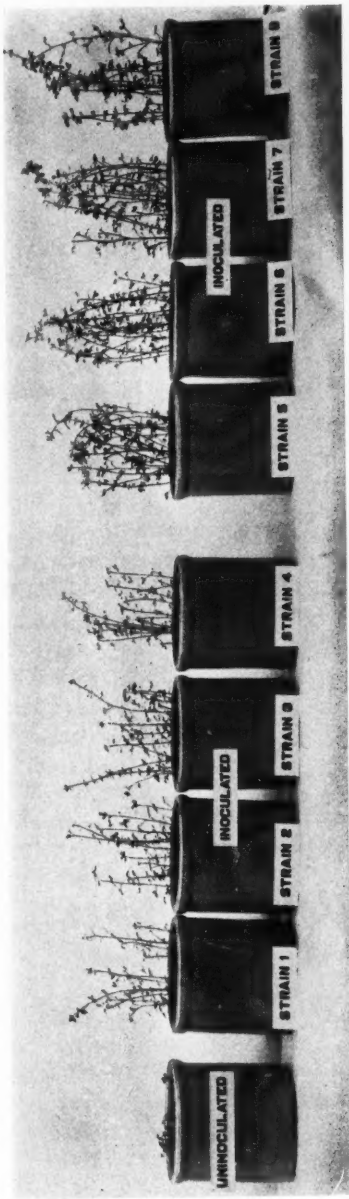
Fig. 1
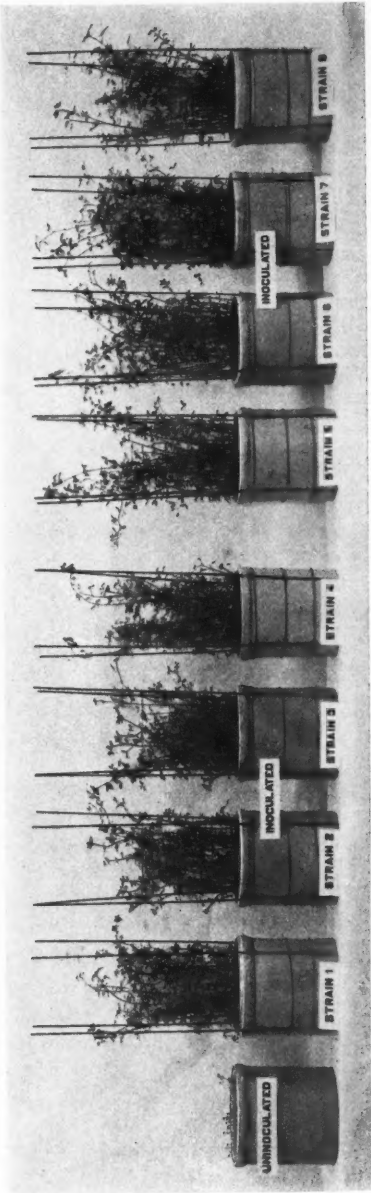
Fig. 2

HEAT OF WETTING OF SOILS DRIED AT DIFFERENT TEMPERATURES AND THE FORCE AT WHICH SOILS ABSORB WATER

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In previous communications (2) the heat of wetting has been proposed as a desirable method for estimating the colloidal material in soils. Since the amount of heat evolved upon wetting is a function not only of the quantity of colloidal material present, its state of activation and its degree of reactivity or affinity for the liquid but also, of the state of its dryness, it has appeared advisable to investigate the heat of wetting of soils dried at different temperatures and thus ascertain definitely at what temperature the soils must be dried to give the most reliable estimation of the colloidal content. Such an investigation, it was also believed, would throw much light on many important and interesting points, such as the actual quantity of water involved in the heat produced, the affinity of different soils or their colloids for water, and the force with which the different soils or their colloids adsorb and compress the water. In this report, therefore, are presented the results obtained from the investigation in question.

PROCEDURE

The soils whose heat of wetting was to be determined were first placed in a saturated atmosphere and allowed to stand therein for several days in order to adsorb vapor moisture and come to equilibrium. Then approximately equal portions of them were dried for 24 hours at the temperatures of 50°, 75°, 107° and 140°C. Their heat of wetting was then determined according to the method previously described (1).

From each soil which had attained equilibrium in the water-saturated atmosphere, a sample was also taken for moisture determination. These samples were dried successively at the same time, at the same temperature and for the same length of time as the samples for the heat of wetting determination, thus making the results in both cases very comparable.

In table 1 are presented the results obtained on the heat of wetting of several types of soils dried at the different temperatures. To the right of the same table is shown the relative percentage of loss of moisture, the loss at 50° being taken as 100 per cent. In table 2 are presented data on the percentage of moisture lost from the soils by successive drying at different temperatures.

The results in table 2 reveal many important and interesting facts. In the

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first place, they show that the heat of wetting is comparatively very small when the soils are dried at only 50°C.   It is increased appreciably when they are dried at 75°, but it receives the greatest increase when the drying temperature of 107° is reached.   Beyond this temperature, even at 140°, the increase is

TABLE 1

Heat of wetting of soils dried at different temperatures

SOILS	RISE OF TEMPERATURE OF 100 GM. SOIL IN 125 GM. WATER. SOIL DRIED 24 HOURS AT				RELATIVE HEAT OF WETTING OF SOILS DRIED FOR 24 HOURS AT			
	50°	75°	107°	140°	50°	75°	107°	140°
	°C.	°C.	°C.	°C.	per cent	per cent	per cent	per cent
Rhode Island sandy loam............	0.916	1.380	2.690	2.730	100	150.7	293.7	298.0
Ohio silt loam.....................	0.773	1.370	1.964	2.000	100	177.3	253.6	258.9
Michigan silt loam.................	0.627	1.852	2.390	3.128	100	295.3	381.3	498.8
Illinois clay loam.................	0.702	2.460	3.727	3.810	100	350.0	530.6	541.2
Minnesota Carrington clay loam......	0.998	3.292	6.030	7.020	100	329.8	604.0	703.2
Michigan Saginaw clay loam........	0.939	3.540	6.820	6.970	100	377.0	726.3	742.4
California yolo clay................	0.774	2.137	4.060	4.490	100	275.3	524.6	580.0
Wisconsin superior clay.............	0.527	1.543	2.830	3.120	100	292.3	537.0	592.0
Michigan shell clay................	0.807	2.140	4.280	4.430	100	265.2	530.3	549.0
Muck..............................	4.625	6.420	27.080	28.400	100	138.8	583.6	614.0
Fuller's earth.....................	1.440	4.490	15.760	16.950	100	311.8	1094.0	1174.0

TABLE 2

Moisture lost from soils by successive drying for 24 hours at temperatures indicated

	TEMPERATURE			
	50°	75°	107°	140°
	per cent	per cent	per cent	per cent
Rhode Island sandy loam......................	2.187	0.680	0.6330	0.511
Ohio silt loam...............................	3.417	0.338	0.3720	0.356
Michigan silt loam...........................	4.280	0.628	0.3700	0.315
Illinois clay loam...........................	5.905	1.492	0.4980	0.485
Minnesota carrington clay loam................	5.340	2.750	1.0080	0.792
Michigan Saginaw clay loam...................	5.590	2.090	0.7193	0.721
California yolo clay..........................	4.780	1.013	0.4620	0.461
Wisconsin superior clay.......................	4.400	0.949	0.3740	0.353
Michigan shell clay...........................	3.745	1.112	0.6030	0.329
Muck.......................................	14.270	6.130	3.4200	4.250
Fuller's earth...............................	11.140	3.880	2.0400	0.805

practically negligible.   If the rise of temperature at 50° is taken as 100 per cent the increase in heat of wetting from 50° to 75° is 150.7 per cent, from 75° to 107° it is 293.7 per cent and from 107° to 140° it is 298 per cent in the case of Rhode Island sandy loam.   In Michigan Saginaw clay loam the increase is 377.0 per cent from 50° to 75°, 726.3 per cent from 75° to 107° and 742.4 per

cent from 107° to 140°. In fuller's earth the increase is 311.8 per cent from 50° to 75°, 1094.0 per cent from 75° to 107° and 1174.0 per cent from 107° to 140°.

It is evident, therefore, that the heat of wetting increases very rapidly as the drying temperature rises, making the greatest increase from 75° to 107° and reaching the limit at around 107°.

Table 2 which contains loss of moisture from the soils at the different drying temperatures reveals equally important and interesting facts. It will be seen at once that the major portion of the moisture is lost at 50°C. and the next largest amount is from 50° to 75°. The amount lost between 75° and 107° and between 107° and 140° is comparatively small, being about ½ per cent excepting muck and fuller's earth. It is interesting to note that moisture lost between 107° and 140° is practically the same as that between 75° and 107°.

The importance and significance of the results contained in table 1 and table 2, are revealed only when both tables are compared. It has already been seen that the greatest heat of wetting is produced when the soils are dried at 107°, yet the amount of moisture expelled at this temperature above that expelled at 75° is very small. Thus, in Michigan Saginaw clay loam the moisture expelled at 107° is only 0.7193 per cent above that expelled at 75°, yet the heat of wetting increased to 3.28°C. above that at the drying temperature of 75°. In other words, the expulsion of 0.7193 per cent moisture raised the temperature of 100 gm. of soil in 125 gm. of water by 3.28°. In the same soil the expulsion of 5.59 per cent moisture at the drying temperature of 50° raised the heat of wetting by only 0.939° and the expulsion of 3.09 per cent moisture at 75° increased the heat of wetting by 2.601° above that at 50°. In fuller's earth the expulsion of 2.67 per cent moisture at the temperature of 107° increased the heat of wetting by 11.27° above that at 75°C. The loss of 17.24 per cent of moisture at 50° raised the heat of wetting to only 1.44° and the loss of 4.5 per cent moisture at 75° above that lost at 50° increased the heat of wetting by only 3.05°. The results in muck and in the other soils are just as striking. They go to show that the greatest amount of heat of wetting is produced by the last traces of moisture, which are extremely small and are expelled at about 107°, as the drying above this temperature does not increase the heat of wetting produced.

Another fact which the general results on heat of wetting reveal, is the high temperature to which the soils would be raised if only that portion of the water which produces the heat of wetting were added to the soil. For instance, the total amount of water in Michigan Saginaw clay loam which is effective in producing the heat of wetting is only 3.81 gm. in 100 gm. of soil. These 3.81 grams of water, when absorbed, raised the temperature of 100 gm. of soil and 125 gm. of water to the extent of 6.82°C. If only the 3.81 gm. of water were added to the 100 gm. of dry soil, the heat of wetting would be raised, according to these results, more than 223.6°. In the case of the muck and fuller's earth if only the effective moisture were added, the heat of wetting would

be raised to 281.0° and 251.3°, respectively, taking 12.10 per cent of effective moisture in producing heat of wetting for muck and 7.80 per cent for fuller's earth. These are certainly tremendous amounts of heat of wetting.

The question raised by the general results on heat of wetting is: Is the heat of wetting due to a physical or chemical cause, or to both? That is to say, is it due to a physical condensation of the moisture, or to a chemical hydration, or both? The results themselves unfortunately do not throw any definite light upon the subject. The only portion of the results which would tend to indicate that it is probably due to a physical cause are those obtained at the temperature of 140°. It is seen that in heating the soils beyond 107° or up to 140° the loss in weight is still appreciable—in fact in most cases it is as great as that which occurred between the temperatures of 75° and 107°—while the heat of wetting is practically the same as that at 107°. It is reasoned from these facts that if the loss in weight at the temperature of 140° represented loss of water, it would probably be water of combination, and not water of physical adsorption but, since this loss produced no heat of wetting, it represents most likely volatilization of the organic and inorganic matter of the soils and not loss of water.

If the heat of wetting is caused by physical phenomena, such as adsorption or condensation of the water on the surfaces of the particles, then some highly interesting facts are revealed. It has already been seen that the amount of moisture which is responsible or effective in producing the heat of wetting is comparatively small. If this effective moisture were added suddenly to the soil, the temperature or heat of wetting would be raised greatly: in Saginaw clay loam it would be raised to 167.8°C., in fuller's earth, to 251.3°, and in muck, to 281°. These high temperatures mean a great change of some sort. If the cause of the heat of wetting is physical, the moisture is probably simply adsorbed and the attraction of the soil for the water causes it to become greatly compressed, or to change its stage of aggregation from the liquid to the solid or semi-solid state, or to a liquid of much greater density than before.

The significance of these high temperatures of heat of wetting lies in the fact that they tend to reveal with what great force the water is adsorbed by the soils and the pressure with which the water films become compressed by the force of attraction. An estimation of these values is afforded by the fact that when water is suddenly compressed the temperature is raised only 0.018° for every 10 atmospheres pressure.[1] Knowing this value and also the temperature of heat of wetting and the amount of water which is effective in producing the heat of wetting, the force of adsorption can be readily calculated. Table 3 presents the results of such calculation for several different types of soil.

It will be seen at once from table 3 that the force with which the soils adsorb and probably compress the moisture films, is really enormous. It ranges from

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[1] These values were kindly supplied by Dr. G. K. Burges, Director of U. S. Bureau of Standards.

92,250 atmospheres in Rhode Island sandy loam to 156,200 atmospheres in muck.

The foregoing results also go to show that the different soils possess a varying degree of affinity for water and adsorb and compress it with different degrees of force.

The data in tables 1 and 2 relating to the heat of wetting and loss of moisture at different temperatures of drying, lead to the practical conclusion that for an accurate determination of the colloidal content of soils, the latter must be dried at a temperature around 107° for more than 10 hours. Certain studies which were conducted to ascertain the length of time that was necessary to dry the soils at 107° in order to bring them to equilibrium, showed that it required more than 10 hours. If the soils were dried for only 5 hours, for instance, the heat

TABLE 3

Effective moisture in producing heat of wetting and force of adsorption

	EFFECTIVE MOISTURE IN PRODUCING HEAT OF WETTING PER 100 GM. SOIL	HEAT OF WETTING IF ONLY EFFECTIVE MOISTURE WERE ADDED TO 100 GM. SOIL	FORCE WITH WHICH EFFECTIVE WATER IS ABSORBED AND PROBABLY COMPRESSED
	gm.	*°C.*	*atms.*
Rhode Island sandy loam......................	2.02	166.3	92,250
Ohio silt loam.................................	1.40	175.0	97,230
Michigan silt loam............................	1.70	176.0	97,800
Illinois clay loam.............................	2.60	179.3	99,560
Minnesota Carrington clay loam...............	4.50	167.8	93,280
Michigan Saginaw clay loam...................	3.81	223.6	124,300
California yolo clay...........................	2.67	190.0	105,600
Wisconsin superior clay.......................	2.00	176.5	98,060
Michigan shell clay...........................	2.30	232.6	129,300
Muck..	12.10	281.0	156,200
Fuller's Earth................................	7.80	251.3	139,600

of wetting of 100 gm. of muck in 125 gm. of water would be only 16.18° as compared with 21.91° when dried for 10 or more hours. This difference was found in almost all the soils.

SUMMARY

A study was made to determine the heat of wetting of soils dried at different temperatures.

Drying soils at 50°, 75°, 107° and 140°C. for 24 hours, caused the heat of wetting to increase rapidly up to the temperature of 107° and to reach its limit at about this temperature. The heat of wetting of soils dried at 107° was from 293.7 to 1094.0 per cent greater than that at the temperature of 50°, the heat of wetting at 50° being taken as 100 per cent.

The amount of moisture which is effective in producing the heat of wetting is comparatively small, ranging from 1.40 per cent to 12.10 per cent. And of

this total effective moisture only a very small part, driven off at 107°, is responsible for the major portion of the heat of wetting.   For instance, in some soils the expulsion of 0.7193 per cent of water at 107°C. raised the temperature of 125 gm. of water in 100 gm. of soil, more than 3.28°C., which is about 50 per cent above that at the drying temperature of 75°C.

If it were possible to add again to the dry soil only its effective moisture content which is responsible for its heat of wetting, the temperature rise would be enormous.   In Saginaw clay loam for instance, the effective moisture content is 3.81 gm. per 100 gm. of soils.   If this moisture were again added to the 100 gm. of soil dried at 107°, the temperature rise would be 223.6°C.

If we assume that the heat of wetting is due mainly to the adsorption of the water film by the soil, and that this water film is probably compressed into a solid or liquid of greater density, the force of adsorption amounts to an enormous value, ranging from 92,250 atmospheres in Rhode Island sandy loam to 124,300 atmospheres in Carrington clay loam, to 156,200 atmospheres in muck.

Experiments have shown that the soils must be dried at around 107°C. for more than 10 hours in order that they may be dried thoroughly and thereby allow a truer estimation of their colloidal content.

Data accumulated seem to indicate that a portion of the loss in weight when soils are heated much above 100°C. represents loss of organic and inorganic matter by volatilization.

REFERENCES

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INOCULATING SOIL WITH AZOTOBACTER[1]

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INTRODUCTION

The very rapid loss of nitrogen from virgin soils when brought under culti-vation, accompanied, as this loss is, by a corresponding decrease in produc-tivity, makes every factor that might possibly compensate for this depre-ciation very important from a soil fertility standpoint.

Ever since the discovery of the existence in soils of non-symbiotic micro-organisms capable of fixing free atmospheric nitrogen, the question of their economic importance as a factor in the nitrogen balance of cultivated soils has been frequently raised. However, most of the research work that has been conducted with these organisms has been of a morphological and physio-logical rather than of an economic nature. There are on record only a few isolated instances of efforts being made to determine their importance in the nitrogen economy of soils.

It is quite generally accepted that among the free-living, nitrogen-fixing organisms thus far studied those belonging to the Azotobacter group are probably of the most importance from the point of view of fixing nitrogen under average soil conditions. The existence of organisms of this group under a variety of field conditions and their ability to fix nitrogen under laboratory conditions have been definitely established. There is also some evidence to indicate that the quantity of nitrogen fixed under field conditions may be quite marked provided suitable conditions for their activity exist. There are, however, many cultivated soils in which Azotobacter do not exist. This fact has led certain investigators to attempt introducing this group, or mem-bers of this group, into soils in which they are not normally found. Many of the earlier efforts along this line were not entirely satisfactory, probably because of the lack of information relative to the conditions essential for the existence and growth of Azotobacter. Much information regarding the physiological characteristics of this group of organisms has been accumulated during the past few years. In the belief that recent investigations have established the fundamental conditions necessary for the existence of these organisms in the average soil, it was thought desirable to undertake some inoculation experiments in which these conditions were fulfilled. Experi-ments of this nature have been in progress for a number of years, and the results

[1] Contribution No. 66, Department of Bacteriology, Kansas State Experiment Station.

of some laboratory experiments have been reported (2).   Certain field experiments have been in progress long enough, it is believed, to justify a preliminary report of the results thus far secured.

The objects of these experiments were: (*a*) To determine the longevity of Azotobacter when introduced under field conditions into soils normally not containing them.   (*b*) To study the effect of altering the reaction of such soils in such a way as to reduce their acidity, upon the longevity of introduced Azotobacter.   (*c*) To determine the effect of introducing Azotobacter into soil, normally not containing them, upon the nitrogen-fixing ability of the soil.   Altogether six series of field experiments have been under observation, but the results of only two typical ones will be reported in this paper.

<div align="center">METHODS</div>

<div align="center">*Preparing plots*</div>

The two series of plots discussed in this paper will hereafter be known as "Series I" and "Series II."   Series I was located on typical upland pasture soil classed as "Derby silt loam."   The reaction of this soil, approximately pH 5.5, is characteristic of large areas of similar soils in this vicinity, none of which exhibits Azotobacter when cultured in a mannite solution.

FIG. 1. ARRANGEMENT OF PLOTS IN SERIES I

The plots in this instance were only 1 foot square and separated by a distance of 1 foot.   The arrangement of the plots is shown in figure 1.   The various additions to be made were added and mixed into the surface 8 inches of soil as well as possible without removing the soil.   No further treatment of any kind was given to any plot.   The treatments were as follows:

Plot 1. Check.
Plot 2. Inoculated with soil containing typical Azotobacter.
Plot 3. Inoculated as in 2; $CaCO_3$, 10,000 lbs. per acre.
Plot 4. $MgCO_3$, 10,000 lbs. per acre.
Plot 5. NaOH, 1,000 lbs. per acre in solution.
Plot 6. $CaCO_3$, 10,000 lbs. per acre.
Plot 7. Inoculated as in 2; $MgCO_3$ 10,000 lbs. per acre.
Plot 8. Inoculated as in 2; NaOH 1000 lbs. per acre in solution.
Plot 9. Check.

The check plots received no treatment other than the initial cultivation. The inoculum consisted of 400 gm. of a neutral soil that always exhibits a typical Azotobacter flora when cultured in a mannite solution. $CaCO_3$ and $MgCO_3$ were added at the rate of 10,000 pounds per acre.   Sodium hydroxide was added at the rate of 1000 pounds per acre in a 10 per cent solution.   The plots were treated October 16, 1919.

Series II was located on bottom land classed as "Wabash silt loam," which normally would be approximately neutral or slightly alkaline. However, this was planted to pine trees a number of years ago and as a result of the decomposition of pine needles, it has become very acid, approximately pH 4.0, in fact the most acid soil yet located in this vicinity. It was because of this very acid condition that it was used for experimental purposes.

June 24, 1920, an area about 12 feet square, free from pine trees, was marked off into 12 areas, each 2 feet square and separated by an alley 1 foot wide, as indicated in figure 2. After the surface covering of pine needles had been removed, the soil from each plot to a depth of 8 inches was trans-
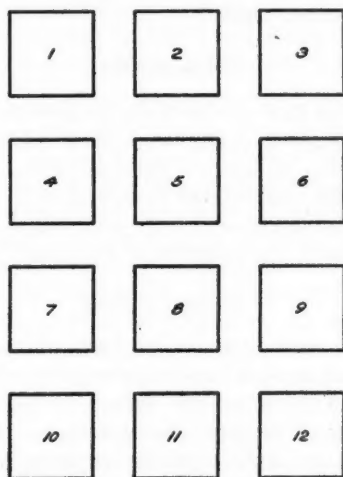
FIG. 2. ARRANGEMENT OF PLOTS IN SERIES II

ferred to a tray, treated as indicated below, thoroughly mixed, and returned, after which the carpet of pine needles was replaced.

Plot 1. Check.
Plot 2. Karo corn syrup 180 gm. in solution.
Plot 3. $CaCO_3$ 420 gm.
Plot 4. Inoculated with soil.
Plot 5. Inoculated with mixed culture.
Plot 6. $CaCO_3$ 420 gm. and inoculated with mixed culture.
Plot 7. Karo 180 gm. and inoculated with soil.
Plot 8. $CaCO_3$ 210 gm. and inoculated with soil.
Plot 9. $CaCO_3$ 420 gm. and inoculated with soil.
Plot 10. $CaCO_3$ 840 gm. and inoculated with soil.
Plot 11. $CaCO_3$ 420 gm. Karo 180 gm. and inoculated with soil.
Plot 12. Check.

Karo syrup has been found to be a very good source of energy for Azotobacter and was added to furnish a readily available source of food. It was added in solution at the approximate rate of 4000 pounds per acre. The $CaCO_3$ was in the form of precipitated powder, and added at rates of 5000, 10,000 and 20,000 pounds per acre. The soil, 1000 gm., used as the inoculum was of the same type, secured only 100 yards away, neutral in reaction, and has never failed to show a typical active nitrogen-fixing flora when cultured in a mannite solution. The mixed culture used as an inoculum was prepared by suspending the films from five 50-cc. mannite cultures inoculated from the same soil as used for the soil inoculum and incubated 7 days at room temperature. All the cultures had a typical Azotobacter film, most of them having already turned black.

Sampling plots

In sampling the plots to be examined for the presence of Azotobacter 200 to 400 gm. of soil were taken, in the case of Series I from two points, and in Series II from one point, by means of sterile spatulas. The surface soil was first scraped off and the sample taken to a depth of 4 to 6 inches. The soil was brought to the laboratory and thoroughly mixed under conditions arranged to prevent outside contamination as far as possible.

Laboratory methods

To 100 cc. of sterile water 50 gm. of the fresh soil was added, thoroughly shaken, and permitted to stand for a moment, after which the supernatant suspension was poured off into a sterile flask. Of this suspension 10 cc. was used to inoculate 50 cc. of mannite cultural solution. The object in pouring the suspension from the soil was to enable one to shake the suspension continuously while transferring, without incorporating the heavier soil particles in the inoculum, thus permitting of a more uniform inoculation. If total nitrogen determinations were to be made, four flasks of media were inoculated, two being immediately sterilized in an autoclave, to act as checks. The inoculated samples were incubated at room temperature for 3 weeks, during the earlier part of the work. This period was later reduced to 2 weeks. Nitrogen determinations were made by the Kjeldahl method on the entire content of the culture flask. During incubation frequent macroscopic examinations were made for an Azotobacter film. If there was any question as to the nature of growth present, small portions of the film were examined for Azotobacter-like organisms under the ¼ objective. In tables 1 and 4, where a + sign has been recorded, either an unmistakable Azotobacter film developed or Azotobacter-like organisms were observed under the microscope.

The pH determinations were made on the air-dried sample of soil, ground to pass a 40-mesh sieve, 14 gm. of which, suspended in 70 cc. of conductivity water, was placed in electrode cells and continuously shaken. Potentiom-

eter readings were recorded until a maximum was reached. From the readings, pH values were obtained from the tables prepared by Schmidt and Hoagland (7). Duplicate determinations were always made and the accuracy of the apparatus checked on a standard acetate mixture prior to each determination.

EXPERIMENTAL RESULTS

As mentioned in the introduction the object of these experiments was threefold. The condensed data bearing upon these three points that have been secured from the two series of experiments under discussion are presented in tables 1 to 6 inclusive.

Because of the marked difference in the reactions of the two soils, the data can perhaps be best analyzed by taking each series separately. The plots of Series I, it should be remembered, are located on a soil with a reaction, of approximately pH 5.5, a reaction not far below that previously reported to be the maximum acidity tolerated by Azotobacter (4). Attention has been called previously (2) to the fact that when Azotobacter are introduced into a soil only slightly more acid than pH 6.0, under laboratory conditions, some time is required for them to disappear. Another fact that is of major significance in analyzing data relative to the longevity of Azotobacter in soils, particularly those that have been limed to alter the reaction, is the difficulty of getting lime or any other insoluble material uniformly distributed in a given mass of soil. Suppose, for example, that a given mass of an acid soil required theoretically 1000 pounds of $CaCO_3$ to reduce the acidity to pH 6.0, and that 800 pounds were introduced and distributed as uniformly as possible. Theoretically the mass of soil would still be acid and in fact analyses would in all probability show an acid condition. However, owing to the difficulty in obtaining a uniform distribution of the $CaCO_3$, there would unquestionably be limited areas within the mass that would react alkaline. Such areas might remain alkaline for months or even years depending upon the mass of $CaCO_3$. A number of instances have been noted in these experiments where particles of $CaCO_3$ were observed in the samples taken for analysis, still the samples actually showed a pH of less than 6.0. Suppose further that a few introduced Azotobacter came to rest in such an area. They would find ideal conditions for their existence, so far as reaction is concerned, and might remain there for months, even years, when the surrounding mass of soil was far too acid for their existence. If such small particles were incorporated in the soil used as an inoculum a typical development of Azotobacter might result from a soil far too acid, as a whole, to support their growth. If these points are kept in mind the data in the accompanying tables can be better understood.

Turning attention for a moment to the data from Series I, it is evident from table 1 (check plots 1 and 9) that this soil does not normally contain Azotobacter. When Azotobacter were introduced with no additional treat-

TABLE 1

Effect of introducing Azotobacter into a soil normally not containing them but treated to support their growth—Series I

PLOT NUMBER	TREATMENT 10/16/19	AZOTOBACTER CULTURES														
		10/16/19	10/24/19	8/11/20	10/5/20	11/18/20	3/9/21	8/2/21	9/8/21	12/30/21	4/6/22	7/18/22	8/10/22	6/16/23	6/5/24	6/24/24
1	Check	− −	− −	+ +	− −	− −	− −	− −	− −	− −	− −	− +	− −	− −	− +	− −
2	Inoculated*	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	− +	+ +
3	Inoculated;* CaCO₃†	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
4	MgCO₃†	− −	− −	+ +	+ +	− −	− −	− −	− −	+ +	− −	+ +	− −	− −	+ +	− −
5	NaOH‡	− −	− −	− −	− −	− −	− −	− −	− −	+ +	− −	− −	− −	− −	− −	− −
6	CaCO₃	− −	− −	− −	− −	+ +	− −	+ +	− −	+ +	+ +	+ +	− −	− −	+ +	+ +
7	Inoculated;* MgCO₃	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
8	Inoculated;* NaOH	+ +	+ +	+ +	+ +	+ +	− −	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
9	Check	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −	− −

* Inoculated with 400 gm. neutral soil containing typical Azotobacter flora.
† CaCO₃ and MgCO₃ added at rate of 10,000 pounds per acre.
‡ NaOH added at rate of 1000 pounds per acre.

ment (plot 2), they were consistently recovered for a period of a year and a half, after which they had apparently disappeared. The three instances in which they were noted after this date can be explained as chance inoculation from adjoining plots. In this instance it required an appreciable length of

TABLE 2

Effect of various treatments upon the reaction of an acid soil—Series I

PLOT NUMBER	TREATMENT 10/16/19	10/24/19	11/18/20	9/8/21	8/10/22	6/16/23	4/14/24	6/24/24
		pH	pH	pH	pH	pH	pH	pH
1	Check	5.39	5.63	5.39	5.41	5.44	5.28	5.36
2	Inoculated*	5.46	5.53	5.36	5.43	5.43	5.43	5.41
3	Inoculated; CaCO₃†	7.78	7.67	7.79	7.62	6.53	7.08	6.94
4	MgCO₃†	8.10	7.88	7.22	7.05	6.53	6.29	6.42
5	NaOH‡	5.97	5.65	5.78	5.63	5.61	5.60	5.24
6	CaCO₃†	7.90	7.74	7.45	7.15	7.34	6.88	6.77
7	Inoculated;* MgCO₃†	7.95	7.50	7.44	6.86	6.73	6.32	6.05
8	Inoculated;* NaOH‡	5.82	5.97	5.75	5.65	5.63	5.31	5.46
9	Check	5.53	5.53	5.46	5.36	5.43	5.68	5.38

* Inoculum 400 gm. neutral soil containing typical Azotobacter flora.
† CaCO₃ and MgCO₃ added at rate of 10,000 pounds per acre.
‡ NaOH added at rate of 1000 pounds per acre.

TABLE 3

Effect of introducing Azotobacter into soil of Series I upon its nitrogen-fixing ability

PLOT NUMBER	TREATMENT 10/16/19	RELATIVE NITROGEN FIXATION (CHECKS = 100)										
		10/24/29	10/5/20	11/18/20	8/2/21	4/6/22	7/18/22	8/10/22	6/16/23	6/5/24	6/24/24	Averages
1	Checks	100	100	100	100	100	100	100	100	100	100	100
2	Inoculated*	156	158	212	168	115	193	195	100	92	155	154
3	Inoculated; CaCO₃†	190	146	190	450	262	345	213	210	193	242	244
4	MgCO₃†	77	138	146	....	....	90	87	152	213	118	128
5	NaOH‡	105	117	134	90	100	152	78	123	101	81	108
6	CaCO₃†	62	75	150	226	183	204	203	107	230	210	165
7	Inoculated; MgCO₃†	132	144	208	435	213	211	228	210	197	226	220
8	Inoculated; NaOH‡	146	145	202	438	88	179	241	143	76	84	174
9	Check	100	100	100	100	100	100	100	100	100	100	100

* Inoculated with 400 gm. neutral soil containing typical Azotobacter flora.
† CaCO₃ and MgCO₃ added at rate of 10,000 pounds per acre.
‡ NaOH added at rate of 1000 pounds per acre.

time for introduced organisms to disappear. When inoculation was accompanied by ample liming to establish and maintain a favorable reaction, either with MgCO₃ (plot 3) or CaCO₃ (plot 7) Azotobacter have been detected in every subsequent examination.

TABLE 4

Effect of introducing Azotobacter into a soil normally not containing them but treated to support their growth—Series II

PLOT NUMBER	TREATMENT 6/24/20	AZOTOBACTER CULTURES															
		6/24/20	8/10/20	10/6/20	11/20/20	3/12/21	8/1/21	9/7/21	12/29/21	3/22/22	5/25/22	6/26/22	8/11/22	6/9/23	4/3/24	5/26/24	6/26/24
1	Check	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2	Karo 180 gm.‡	?	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
3	CaCO₃ 420 gm.	++	−	+	+	+	−	+	−	−	−	−	+	+	+	−	−
4	Inoculated (soil)*	++	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
5	Inoculated (culture)†	++	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
6	CaCO₃ 420 gm.; inoculated (culture)†	++	++	++	++	++	++	++	++	++	++	+	++	++	++	+	++
7	Karo 180 gm.; inoculated (soil)*	++	−	−	−	+	−	+	−	+	+	+	−	−	−	−	−
8	CaCO₃ 210 gm.; inoculated (soil)*	++	++	++	++	++	−	++	−	++	?	−	−	−	−	−	−
9	CaCO₃ 420 gm.; inoculated (soil)*	+−	++	++	++	++	−	++	−	−	?	−	+	+	−	−	−
10	CaCO₃ 840 gm.; inoculated (soil)*	++	++	++	++	+	++	++	++	++	+	++	++	++	+	++	−
11	CaCO₃ 420 gm.; Karo 180 gm.; inoculated (soil)*	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
12	Check	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

* 1000 gm. of a neutral soil containing typical Azotobacter flora.

† Films from typical Azotobacter cultures.

‡ Karo 180 gm. = 4000 pounds per acre. CaCO₃ 210 gm. = 5000 pounds per acre. CaCO₃ 420 gm. = 10,000 pounds per acre. CaCO₃ 840 gm. = 20,000 pounds per acre.

In the plot receiving NaOH (plot 8) the introduced organisms were consistently detected for approximately two years; were intermittently noted during the third year; and had apparently disappeared thereafter. An examination of the reaction of this soil, recorded in table 2, will show that while the NaOH somewhat reduced the acidity, the quantity was not sufficient to render the reaction favorable for the existence of Azotobacter, and as the acidity again rose the organisms disappeared.

When the reaction of the soil was made favorable without at the same time introducing Azotobacter (plots 4 and 6) their subsequent detection was very

TABLE 5

Effect of various treatments upon the reaction of a strongly acid soil—Series II

PLOT NUMBER	TREATMENT 6/24/20	6/24/20	3/12/21	8/1/21	6/26/22	6/9/23	5/26/24	6/26/24
		pH	pH	pH	pH	pH	pH	pH
1	Check	3.73	4.07	3.92	4.02	3.97	3.60	3.23
2	Karo 180 gm. ‡	4.02	4.14	4.09	4.41	4.28	4.19	3.65
3	CaCO₃ 420 gm.	6.88	7.00	7.27	6.17	6.07	5.70	6.49
4	Inoculated (soil)*	3.82	4.14	3.97	4.51	3.99	3.63	3.41
5	Inoculated (culture)†	3.94	4.06	4.04	4.26	4.09	3.94	3.80
6	CaCO₃ 420 gm.; inoculated (culture)†	7.49	6.42	5.63	5.76	4.97	5.51	4.90
7	Karo 180 gm.; inoculated (soil)*	3.84	4.19	4.11	4.41	4.24	4.11	3.41
8	CaCO₃ 210 gm.; inoculated (soil)*	6.93	5.12	5.11	5.09	4.90	4.82	4.34
9	CaCO₃ 410 gm.; inoculated (soil)*	6.70	6.70	6.56	5.98	5.63	5.26	4.92
10	CaCO₃ 840 gm.; inoculated (soil)*	7.02	7.41	7.13	7.03	6.74	6.91	6.54
11	CaCO₃ 420 gm.; Karo 180 gm.; inoculated (soil)*	7.08	6.91	6.27	5.43	6.09	6.31	5.88
12	Check	4.01	4.26	4.14	4.38	4.23	4.04	3.58

* 1,000 gm. neutral soil containing typical Azotobacter flora.

† Films from typical Azotobacter cultures.

‡ Karo 180 gm. = 4,000 pounds per acre. CaCO₃ 210 gm. = 5,000 pounds per acre. CaCO₃ 420 gm. = 10,000 pounds per acre. CaCO₃ 840 gm. = 20,000 pounds per acre.

inconsistent. If it be remembered that the presence of Azotobacter in these plots was dependent upon accidental transfer and establishment from adjoining plots, and that this must have taken place at the surface which is almost always dry, it is easily understood that the chances for many organisms gaining entrance are not very great. It might require years to establish a typical flora in this way.

The data from Series II is even more definite and conclusive, probably because of the much stronger initial acid condition, than that from Series I. The results recorded in table 4 (plots 1, 2, and 12) show that this soil normally

will not initiate the growth of Azotobacter in a mannite solution. When Azotobacter are introduced into the soil without at the same time altering the reaction they almost immediately disappear (plots 4, 5, and 7). Even the introduction of a supply of available food in the form of Karo syrup (plot 7) did not enable the organisms to survive in sufficient numbers to be detected at the first examination made six weeks after inoculation.

When $CaCO_3$ was added at the rate of 5,000 pounds per acre accompanied by inoculation (plot 8) Azotobacter were consistently recovered during the

TABLE 6

Effect of introducing Azotobacter into soil of Series II upon its nitrogen-fixing ability

PLOT NUMBER	TREATMENT 6/24/20	RELATIVE NITROGEN FIXATION (CHECKS = 100)									
		10/6/20	11/20/20	8/1/21	5/25/22	6/26/22	8/11/22	6/9/23	4/3/24	5/26/24	Averages
1	Check	100	100	100	100	100	100	100	100	100	100
2	Karo 180 gm.‡	140	92	127	107	90	76	91	78	49	94
3	CaCO₃ 420 gm.	206	105	134	131	125	164	151	185	76	143
4	Inoculated (soil)*	143	105	123	63	100	144	87	105	109	109
5	Inoculated (culture)†	116	94	130	74	80	108	96	60	105	96
6	CaCO₃ 420 gm.; inoculated (culture)†	236	200	346	269	285	304	169	213	251	253
7	Karo 180 gm.; inoculated (soil)*	130	129	135	164	285	60	132	79	141	139
8	CaCO₃ 210 gm.; inoculated (soil)*	271	181	46	41	155	128	112	112	115	129
9	CaCO₃ 420 gm.; inoculated (soil)*	228	200	291	112	140	132	152	160	148	174
10	CaCO₃ 840 gm.; inoculated (soil)*	244	178	304	112	165	232	252	217	173	209
11	CaCO₃ 420 gm.; Karo 180 gm.; inoculated (soil)*	200	160	325	206	365	220	142	282	206	234
12	Check	100	100	100	100	100	100	100	100	100	100

*1000 gm. neutral soil containing typical Azotobacter flora.

† Films from typical Azotobacter culture.

‡ Karo 180 gm. = 4000 pounds per acre. CaCO₃ 210 gm. = 5000 pounds per acre. CaCO₃ 420 gm. = 10,000 pounds per acre. CaCO₃ 840 gm. = 20,000 pounds per acre.

first year and occasionally during the second year after which they were never found. If the presence of Azotobacter be compared with the reaction recorded in table 5, it will be noted that the reaction immediately following treatment was unquestionably favorable, being much less acid than pH 6.0, but that the H-ion concentration soon rose to a point where Azotobacter could not exist. However, several months probably elapsed before all particles of $CaCO_3$ disappeared, thus enabling Azotobacter to survive and be detected during a period of two years.

When the quantity of $CaCO_3$ was doubled, 10,000 pounds per acre added,

accompanied by inoculation (plots 6, 9, and 11) Azotobacter were consistently detected throughout the entire experiment in plots 6 and 11 and in most instances in plot 9. The inoculum used in plot 6, mixed culture, undoubtedly added many more organisms than were added to plots 9 and 11 while the addition of an available food material together with a favorable reaction enabled those introduced into plot 11 to multiply much more rapidly and become more thoroughly established, hence the differences exhibited by somewhat similarly treated plots. Both plots 6 and 9 apparently became too acid for the existence of Azotobacter but as already mentioned, even though the mass of soil is rather strongly acid, small particles of $CaCO_3$
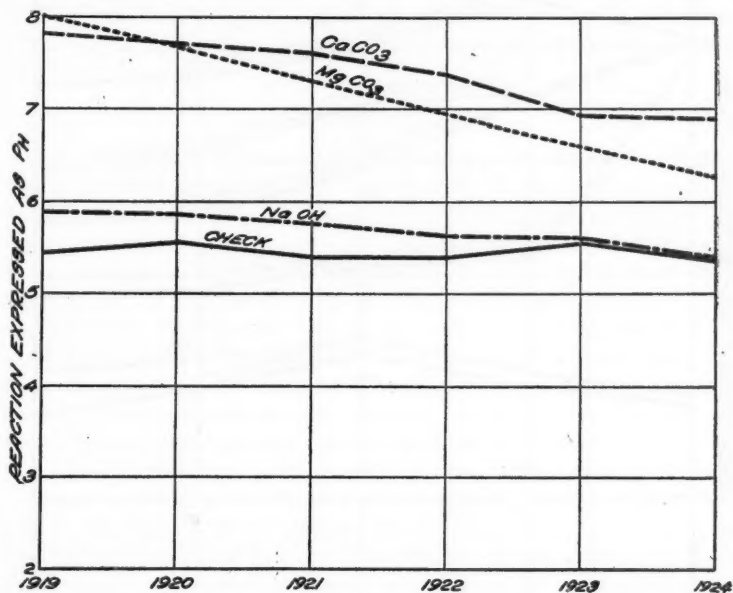

FIG. 3. REACTION OF SOIL FROM PLOTS OF SERIES I WITH TREATMENTS INDICATED

are still visible in areas of the plot, and Azotobacter may be cultured from these plots for some time to come.

Plot 10, inoculated and limed at the rate of 20,000 pounds per acre, still has a reaction near neutral and will probably continue for several years to exhibit Azotobacter consistently.

Where the reaction was made favorable by adding $CaCO_3$ without inoculating (plot 3) Azotobacter have been detected occasionally. The covering of pine needles probably made it more difficult for the organisms to be transferred naturally from one plot to the other than in Series I.

The effect of introducing Azotobacter into soils normally free from them

upon their nitrogen-fixing ability is shown quite definitely in the data presented in tables 3 and 6. As was to be expected quite marked variations were shown by the individual plots and for this reason it has seemed best to arrange the data on a relative rather than absolute basis. After the checks were evaluated at 100 and those plots compared that were variously treated but have exhibited no Azotobacter (plot 5, Series I, receiving NaOH, and plots 2, 4, and 5, Series II, receiving respectively Karo syrup, soil in-
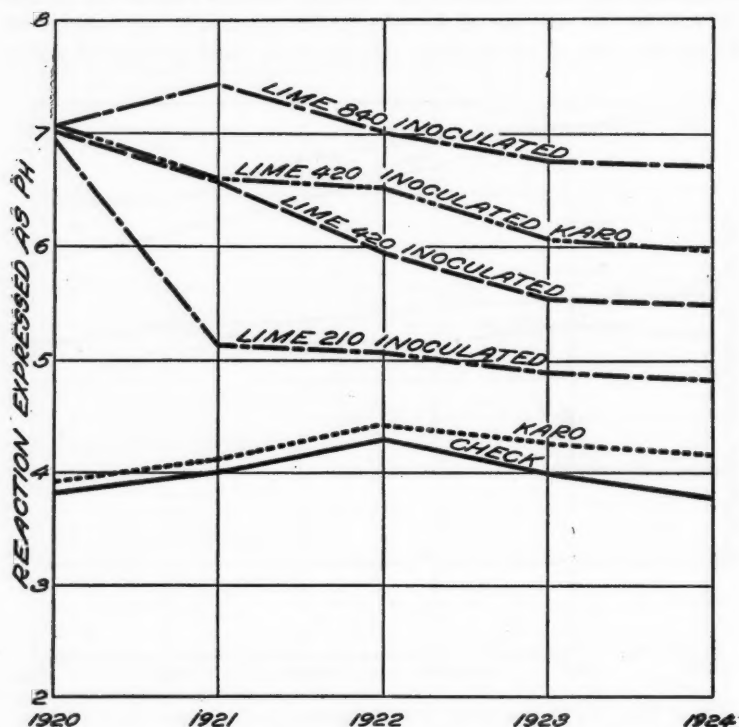

FIG. 4. REACTION OF SOIL FROM PLOTS OF SERIES II WITH TREATMENTS INDICATED

oculum, and culture inoculum), no increase in nitrogen fixation was evident. On the other hand those plots that were so treated as to maintain an Azotobacter flora (plots 3 and 7, Series I and plots 6, 10, and 11, Series II, all limed and inoculated) have shown an average nitrogen fixation of from two to two and one-half times that of the checks. The remaining plots all of which have some time shown Azotobacter, have varied in their relative nitrogen-fixing ability from 128 to 174 per cent of the check, depending upon the frequency with which Azotobacter were cultured.

The effect of the various treatments upon the reaction of the soil is shown graphically in figures 3 and 4. In both these figures the graphs represent the average of all similarly treated plots. In some instances the annual pH value represents the average of two or more analyses, in other instances only one determination was made. It sometimes happened in treated plots that two samples collected within a short time of each other varied considerably in their pH value so that it is not intended that the graphs or data in tables 2 and 5 represent the absolute reaction for any specific time, but rather the general tendency in the reaction of the various plots. No explanation is available as to why the check and Karo treated plots of Series II showed a definite decrease in acidity followed by an increase, unless the decomposition of large quantities of organic matter following the initial cultivation had something to do with it.

DISCUSSION

It is unfortunate that the plots in both series under study were not larger and more widely separated. The large number of samples taken, gradually brought about depressions in the small plots thus causing water to flow from higher to lower points while the short distance apart permitted a more ready natural transfer by rain, etc., of organisms from one plot to another. These factors, together with the difficulty in obtaining uniform distribution of lime and inoculum, undoubtedly account for some of the irregularities in the presence of Azotobacter recorded. Such irregularities have rarely been noted in laboratory experiments. These, however, in no wise screen the fundamental facts emphasized by the data; namely, (a) That the introduction of Azotobacter into soils which normally do not contain them because of a too acid condition, is without effect in establishing a permanent Azotobacter flora; (b) That if the acidity of such soils is reduced to a point favorable to Azotobacter by the addition of such basic materials as $CaCO_3$ and $MgCO_3$, an Azotobacter flora can be established by inoculating; (c) That if the acidity of such treated soils again rises above pH 6.0 the Azotobacter will disappear; and (d) That the nitrogen-fixing ability, as measured by culturing in a mannite medium, of a soil normally devoid of Azotobacter can be markedly increased by establishing an Azotobacter flora.

It is believed that the facts here presented lend substantial proof to former conclusions arrived at in this laboratory, namely, that the absolute reaction of a soil is the primary factor governing its ability to support an Azotobacter flora. It is also believed that these experiments, together with other recently published data from this laboratory (2, 3, 4) offer a satisfactory explanation for many of the irregularities and discrepancies previously reported in inoculation experiments. Take, for example, the inoculation experiments of Lipman and Brown (5). In only a few unrecorded instances were they able to recover Azotobacter one year after inoculation. It is true they added $CaCO_3$ up to 2000 pounds per acre to some of their cylinders but there is no experi-

mental data to show what effect such an addition had upon the absolute reaction of the soil. In view of the data presented in this paper it is almost certain that this quantity was not sufficient to render the reaction favorable to Azotobacter in those cylinders where the introduced organisms could not be recovered. In conclusion Lipman and Brown say:

> They (the experiments) do show that these organisms (Azotobacter) will not survive or remain prominent in soils which do not offer suitable conditions for their growth, and future experiments must be directed toward a better understanding of these suitable conditions.

Likewise in the inoculation experiments of Emerson (1), reported as late as 1918, the conditions essential for the existence of Azotobacter were apparently ignored. While no mention is made of the reaction of the soil studied, the very close correlation reported from this laboratory between the presence of Azotobacter and the reaction, indicate that it was acid. At least some condition existed which inhibited Azotobacter development, otherwise they would have been normally present in the soil. As a result no beneficial effect upon the nitrogen balance of the soil resulted from introducing the Azotobacter.[2] None could be expected in a soil where the fundamental conditions essential for the growth of the organisms did not exist.

On the other hand where the physiological requirements of the organisms are taken into consideration as was done in the recently reported experiments of Makrinoff (6) there is some evidence of very marked beneficial results following Azotobacter inoculation.

It is unfortunate that these experiments were not so planned that the nitrogen balance of the soil could also be studied. Other experiments now under way were planned with this in view and it is hoped that they will give some very definite information with regard to the effect of inoculating with Azotobacter upon plant growth and upon the nitrogen balance of the soil. The experiments here presented indicate very clearly one fundamental condition that must be fulfilled if one wishes to attain success in inoculating Azotobacter-free soils with this group of organisms. There are probably other conditions that must also be taken into consideration, such as the supply of available phosphorus, if success is to be attained in altering the nitrogen balance of a soil by introducing Azotobacter.

CONCLUSIONS

1. A permanent Azotobacter flora could not be established in the two Azotobacter-free soils studied, merely by introducing active living organisms.

2. The absence of Azotobacter from these two soils and the failure of introduced Azotobacter to survive can be attributed to the acid condition of the soil.

[2] This statement is based upon the fact that in approximately 50 per cent of the recorded comparisons between inoculated and uninoculated soils the latter showed a greater gain in nitrogen than did the former.

3. When Azotobacter were introduced into plots to which sufficient basic materials ($MgCO_3$ and $CaCO_3$) were added to make and maintain a favorable reaction, i.e., a H-ion concentration of less than pH 6.0, an Azotobacter flora could be established which, as far as experimental data are available, was permanent.

4. If Azotobacter were introduced together with insufficient basic materials to maintain a favorable reaction they were able to survive for only a limited time after the reaction reverted to a condition more acid than pH 6.0.

5. The establishing of an Azotobacter flora in these soils, normally free from Azotobacter, increased their nitrogen-fixing ability from two to two and one-half times, as measured in these experiments.

6. The data presented in this paper together with those previously published from this laboratory offer a probable explanation for many of the irregular and inconclusive Azotobacter inoculation experiments reported by other investigators.

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CLAYS AS SOIL COLLOIDS

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Since the publication of the paper of Moore, Fry, and Middleton in 1921 (9) on the subject of ultra clay,[1] a considerable number of others have appeared on the properties of inorganic soil colloids and on the methods for their estimation in the soil. The object of the present communication is to lay stress on two important facts which emerge from these results:

(a) The inorganic colloid content of most soils is practically identical with the clay content as determined by mechanical analysis in the ordinary way.

(b) There is a striking relation between the physical and chemical properties and the chemical composition of soil clays and colloids obtained from widely different sources.

THE IDENTITY OF "COLLOID" AND "ORDINARY" CLAY

The early work which refers to the proportion of colloidal matter in soil suggests that only a small percentage of clay is obtainable in the colloid form, and quite recently, G. W. Robinson (11) has stated that even in heavy clays, the colloidal material forms only a fraction of the total material present. On the other hand, W. O. Robinson and Holmes (14) consider that the clay fraction of mechanical analysis is nearly all colloidal; this view was expressed by the writer about the same time (10).

Moore, Fry, and Middleton (9) determined by two methods the amount of colloidal material in a soil which contained 35 per cent of clay, finding it to be 28.3 per cent, which at once suggests the view expressed in (a). Unfortunately, many writers have given results for the amount of colloidal material without those of the mechanical analysis, so that other comparisons are not possible. In this laboratory direct experiments have been made which show that practically the whole of a clay fraction may be obtained in the colloid condition. These experiments were made with clay fractions obtained by sedimentation and highly purified by dialysis as described elsewhere (5), the object being to examine the properties of the non-colloidal portion left after the complete separation of the ultra clay. In order to separate the colloidal matter, a dilute suspension (1 per cent or less) was passed through the Sharples super-centrifuge at 10 to 20 liter per hour, a much more dilute suspension of the ultra

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[1] For the purposes of this paper, the terms "ultra clay" and "colloidal clay" are regarded as synonymous.

clay thus being obtained. This was then separated either by flocculation or by passing it again through the super-centrifuge at the rate of only 1 to 2 liters per hour.

The residue from the first centrifuging (presumably containing the non-colloidal clay) was then well shaken with water and treated in the same way as the original suspension, when it was found to yield a further portion of ultra clay. The process was repeated many times without any sign of the yield of ultra clay becoming exhausted: owing to the nature of the apparatus, the operations could not be carried out quantitatively, a certain amount of material being lost each time. However, one lot of 15 gm. of Gezira clay was treated as many as 47 times, after which the residue amounted to only 2 gm., a steady supply of ultra clay being maintained to the end. This indicates that there is no justification for the customary distinction between colloidal and non-colloidal clay fractions.

In another experiment, slate-blue clay from the Upper Nile was treated 17 times. The amount taken was 16 gm. and after 11 centrifugings had been per-

TABLE 1

Origin, color and plasticity of some materials examined

REFERENCE NUMBER*	ORIGIN	COLOR	PLASTICITY
6640	Gezira	Brown	High
9228	Upper Nile	Slate-blue	Very high
10120	English ball clay	White	Moderate
13107	Subsoil, Mongalla	Red	Very low

* Details as to the origin and chemical composition of these have been given in a recent publication (6).

formed, 58 per cent had been obtained in the form of ultra clay, 29 per cent was left as the "non-colloidal" portion and 13 per cent had been lost. On centrifuging 6 times more, 10 per cent of the residue was obtained as ultra clay and the process showed no signs of coming to an end.

Experiments were also carried out, though not so completely, with an English ball clay and a basic subsoil red clay from the Southern Sudan with precisely the same indications.

Table 1 shows the range of materials examined. The experiments were always carried out with purified fractions of clay (less than 2μ) dimensions.

There is of course, no reason why 2μ should be the limit which divides the colloid from the non-colloid portion and in fact, most ordinary soil clay is much finer than this. Two of the above clays, were kindly examined for the writer by Professor Sven Odén who found that 71 per cent of no. 6640 and 68 per cent of no. 9228 consisted of particles less than 0.4μ in diameter. This is the limit for clay proposed by G. W. Robinson (12) and apart from practical difficulties, would seem to be very satisfactory.

RELATION BETWEEN PHYSICAL AND CHEMICAL PROPERTIES AND CHEMICAL COMPOSITION

As far as ordinary clays are concerned, evidence has been put forward (6) for the view that there is a relationship between many of the properties of a group of clays of similar origin and the chemical composition for which the criterion is the silica-alumina ratio. For soil colloids this view has not always been held: thus W. O. Robinson (13) concluded that the water-absorbing power of 34 soil colloids of different origins was nearly constant. In fact, however, the amounts of water absorbed per gram of colloid ranged from 0.24 to 0.34 gm., and an examination of the figures for 24 of them for which the chemical analyses are given in a subsequent publication (14), points to a relationship (often obscured in individual cases) between absorptive capacity and

TABLE 2

Some colloids grouped according to silica-alumina ratio

NUMBER OF SAMPLES	SILICA-ALUMINA RATIO		WATER ABSORPTION	
	Range	Mean	Range	Mean
4	Over 4	4.23	348–308	324
6	Between 3 and 4	3.43	337–294	310
15	Between 2 and 3	2.42	320–241	277

TABLE 3

Properties, composition and heat of wetting of some soil colloids

REFERENCE NUMBER OF COLLOID*	SOURCE	SILICA-ALUMINA RATIO	WATER ABSORPTION	TENSILE STRENGTH	HEAT OF WETTING
35	Sharkey clay	4.14	308	57.6	16.3
23	Marshall silt loam	3.58	294	47.8	14.2
27	Norfolk fine sandy loam	2.09	243	32.5	6.0

* Analyses given by Holmes and Robinson (14).

chemical composition. In table 2 the 24 colloids are divided into 3 groups according to their silica-alumina ratio.

Further information as to the relation between properties and composition can be obtained from the papers of Middleton (8) on the binding power, and of Anderson (1) on the heat of wetting of soil colloids. It is assumed that these workers used the same materials for which the analysis is given by Holmes and Robinson (14). In the case of 3 colloids, data are available for the water absorption, the heat of wetting and the tensile strength of briquettes made with 25 per cent colloid and quartz flour.

One of the properties which has been studied here is that called "the imbibitional" water-holding capacity, as described by Fisher (4), which is the excess of water retained by a material above that which is held in the interstices between the solid particles. Fisher measures it by observing the mois-

ture equivalent in the ordinary way with water and making a second deter-
mination using xylene. The volume of xylene retained gives the interstitial
pore space, and that of the water gives the imbibitional and interstitial
water together: the difference is the imbibitional power. The results in table
4 were obtained for 6 Sudan soil clays and for comparison are added the
rates of evaporation for the same substances measured by the method of
Keen (7): these of course vary with the moisture content of the clay and are
given as percentage of water lost per minute at 35°C. when the moisture con-
tent is 10 per cent.

With the exception of the rate of evaporation from one clay, the correlation
of composition and properties is satisfactory.

TABLE 4

Imbibitional water-holding capacity and rate of evaporation of 6 Sudan soil clays

REFERENCE NUMBER[*]	ORIGIN	SILICA-ALUMINA RATIO	IMBIBITIONAL WATER	RATE OF EVAPORATION
7900	Red soil	2.85	44	0.202
8320	Brown alluvium	3.77	79	0.178
6640	Brown loess	3.90	101	0.072
9223 }	Brown alluvium	3.93	111	.....
6960 }		3.95	118	0.093
9228	Blue loess	4.79	120	0.054

[*] Details as to the origin and chemical composition of these have been given in a recent
publication (6).

TABLE 5

Some properties of Orangeburg fine sandy loam and Wabash silt loam soil colloids

	ORANGEBURG	WABASH
Silica-alumina ratio..................................	2.200	4.290
Binding power (8)....................................	33.000	154.200
Heat of wetting (1)...................................	....	17.600
Water absorption (13)................................	263.000	333.000
Imbibitional water...................................	79.000	134.000
Evaporation rate at 10 per cent moisture..............	0.151	0.108

In conclusion some figures are presented in table 5 for two colloidal prep-
arations[2] from the Orangeburg fine sandy loam and the Wabash silt loam
soils, listed by Robinson and Holmes (14, p. 14) as nos. 31 and 43 respectively.
The last two sets of figures were obtained in this laboratory.

There are difficulties in extending this generalization: it is obvious that
there may be highly basic silicates (in which the ratio of silica to alumina is
less than 2) possessed of clay-like properties to a considerable extent and the
proposition can be applied successfully only to a group of materials of similar
origin and general chemical composition.

[2] The writer is indebted to Mr. C. R. N. Strouts for the measurements of the rates of
evaporation of water from the different clays, and to Professor Milton Whitney for supply-
ing the Orangeburg and Wabash colloidal preparations.

EXTENT TO WHICH CLAY IS HOMOGENEOUS

The view that ordinary clay and ultra clay are for practical purposes identical raises the question as to whether the former is homogeneous. This can be answered only by an examination of the results of anlayses of the products of fractionation of ordinary clays. In the examples quoted in table 6, the molecular ratio of silica to alumina is taken as sufficient indication of chemical composition for this purpose.

TABLE 6

Molecular ratio of silica to alumina as an indication of chemical composition

REFERENCE TO LITERATURE OR MATERIAL	MOLECULAR RATIO OF SILICA TO ALUMINA
Bradfield (3, p. 20)...............	Suspensoid, 3.54; emulsoid, 3.14
Robinson and Holmes (14, p. 19):	
No. 18........................	First fraction, 2.25; third fraction, 3.30
No. 23........................	First fraction, 3.47; fourth fraction, 4.37
Blanck and Preis (2, p. 73).........	First fraction, 1.90; fifteenth et seq., 2.41
Sudan clay no. 6960..............	Whole clay, 3.95; ultra clay, 4.54
Sudan clay no. 10195.............	Whole clay, 3.90; ultra clay, 4.20

TABLE 7

Dye absorption and chemical reactivity of 2 samples of ultra clay

		SILICA-ALUMINA RATIO	DYE ABSORPTION	DECREASE OF pH
			per cent	
No. 6960	Whole...............	3.95	29	....
	Fine................	4.54	72	....
	Coarse..............	....	..	Slight increase
No. 10195	Whole..............	3.90	35	....
	Fine................	4.20	41	1.03

In the last two cases, the ultra clay analysed was that obtained in the early centrifugings: the further the process is carried out, the nearer of course, would be the composition of the ultra clay to that of the whole clay.

The range of composition is in no case large, but would be sufficient to account for a difference in the chemical and physical properties of the finer and coarser fractions. It is most desirable that further information should be collected on this question and this is being attempted here. Meanwhile a few isolated observations may be recorded for the two materials at the end of table 6. The figures in table 7 give the methylene blue absorption determined according to the method described in the appendix to (5) and the chemical reactivity as indicated by the change of pH on the addition of 0.1 per cent calcium sulfate.

SUMMARY

Repeated centrifuging in a super-centriuge and shaking of clay suspensions show that practically the whole of a clay fraction may be obtained in the colloid condition. Several clay samples were investigated in this manner.

There is a certain correlation between the physical and chemical properties and the chemical composition of clays. The property investigated was the imbibitional water-holding capacity of clay, 6 Sudan soil clays being thus examined.

A comparison of the molecular ratios of silica to alumina in the various fractions of ordinary clay was made.

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